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Section D
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Continuing the activities of the
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BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

יוצא לאור ע"י

מוסד ויצמן לפרסומים במדעי הטבע ובטכנולוגיה בישראל

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CONTENTS

Our gratitude is due to Mrs. Jeanne Beckman of White Plains, New York for enabling us through her generous gift to carry out the work published in the following papers: "The influence of gibberellic acid and kinetin on germination and seedling growth of lettuce" (p. 65) and "The respiration of lettuce seeds germinated at a high temperature" (p. 108).

THE INFLUENCE OF GIBBERELLIC ACID AND KINETIN ON GERMINATION AND SEEDLING GROWTH OF LETTUCE

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ABSTRACT

Kinetin in various concentrations (10^{-5} — 10^{-4} M) stimulates the germination of lettuce seeds var. Grand Rapids in the dark. It does not affect hypocotyl growth but inhibits root growth.

If red light and kinetin are applied simultaneously there is an additive stimulation of germination. Far red does not reverse the stimulation caused by kinetin. It is concluded that kinetin and red light stimulate germination by different mechanisms.

The inhibition of root growth caused by kinetin is not affected by red light.

Gibberellic acid stimulated germination in the dark at concentrations of 2.9×10^{-5} and 2.9×10^{-4} M. It did not affect root or hypocotyl growth. If gibberellic acid is given in conjunction with red light there is an additive stimulation of germination. Far red reverses part of the stimulation caused by gibberellic acid.

The presence of gibberellic acid does not change the sensitivity of the seeds toward red light.

Experiments of transfer from water to gibberellic acid and vice versa at different times after the seeds were put in water make it probable that gibberellic acid affects germination through at least two different processes.

Gibberellic acid did not affect the growth of excised roots either in darkness or light. The growth of excised hypocotyls was not affected by gibberellic acid either. But there was a stimulation, when gibberellic acid was given simultaneously with white or red light to entire seeds or seedlings.

The effect of both kinetin (K) and gibberellic acid (G. A.) on the germination of lettuce seed has recently been investigated (Miller 1956, Kahn et al. 1956 and 1957, Lona 1956). These workers report that the germination stimulating effect of these compounds differs from the stimulation caused by red light, in not being reversible by far-red. In addition there is an ever growing list of reports in the literature on the effect of kinetin and the gibberellins, on the enlargement and growth of various organs and tissue sections (Brian and Hemming 1955; Brian, Hemming and Radley 1955; Das, Patau and Skoog 1956; Marth, Audia and Mitchel 1956; Lang 1956; Wickson and Thimann 1956).

Received July 31, 1957.

The aim of this study was to investigate in greater detail the effect of K and G. A. on growth and germination of lettuce seeds and seedlings. In addition the relationship of the effect of the compounds to that of red (R) and far-red (FR) radiation was investigated.

METHODS

Kinetin solutions were prepared in dilute KOH and brought to *p*H 6 with HCl. G. A. was dissolved in hot water and then neutralized with dilute NaOH. The germination tests were carried out in Petri dishes on filter paper in the dark at 26°C. Illumination with R and FR was given from an incandescent lamp, the light intensity of which was 250 f. c. at the level of the seeds, filtered through appropriate filters. (Evenari and Neumann 1953). Unless otherwise stated R was given for 2 min., 2 hours after the seeds were put in water, the reversing FR being given immediately afterwards. If FR alone was given it was applied 20 mins. after placing the seeds in water.

Lettuce seeds var. Grand Rapids were used throughout the experiments. The effect of various concentrations of the two substances was tried on two lots of seeds: (a) seeds bought from the Ferry Morse Seed Growers, California, (b) locally grown seeds. All subsequent experiments were carried out with lot (b), only. Germination counts and length measurements were made after 72 hours.

RESULTS AND DISCUSSION

The effect of varying concentrations of kinetin and G. A. on germination in darkness and on the length of roots, and hypocotyls of the seedlings is given in Tables I and II. It will be seen that for kinetin the stimulation of germination was highly significant but the differences between the various concentrations were not significant.

TABLE I

Effect of kinetin on germination and growth of lettuce seeds germinated in darkness. Numbers in brackets give the standard deviation

		Molar concentration of kinetin				
	Lot	0	10^{-5}	2.5×10^{-5}	5×10^{-5}	10^{-4}
Germ. %	a	37.3 (12.7)	93.8 (1.0)	84.8 (5.7)	86.3 (4.8)	94.8 (2.1)
	b	3.0 (4.0)	52.5 (6.8)	36.5 (1.2)	27.5 (1.5)	41.0 (14.3)
Root length (mm)	a	17.7 (1.5)	11.8 (0.8)	15.4 (1.9)	16.9 (1.0)	4.9 (0.5)
	b	14.2 (2.8)	7.5 (1.6)	8.7 (4.4)	10.4 (2.6)	3.0 (0.2)
Hypocotyl length (mm)	a	7.0 (1.6)	6.4 (0.4)	5.9 (0.6)	6.4 (1.0)	6.5 (0.6)
	b	9.5 (2.4)	5.4 (0.4)	6.7 (1.4)	7.5 (1.8)	4.3 (0.8)

TABLE II

Effect of gibberellic acid on germination and growth of lettuce seeds germinated in darkness. Numbers in brackets give standard deviation

	Lot	Molar concentration of gibberellic acid 0	2.9×10^{-8}	2.9×10^{-7}	2.9×10^{-6}	2.9×10^{-5}	2.9×10^{-4}
Germ. %	a	28.3 (10.9)	29.3 (9.0)	29.3 (8.5)	40.8 (13.6)	78.0 (25.7)	93.0 (1.0)
	b	12.2 (5.6)	14.0 (4.8)	15.0 (7.9)	19.3 (6.8)	32.0 (10.2)	78.5 (3.5)
Root length	a	16.8 (3.9)	18.2 (5.0)	16.3 (8.0)	15.2 (4.0)	16.0 (4.0)	18.2 (0.3)
(mm)	b	15.0 (3.0)	13.7 (4.0)	16.0 (2.1)	13.7 (1.7)	15.0 (3.3)	17.0 (3.0)
Hypocotyl	a	10.0 (3.3)	10.8 (2.4)	11.3 (3.0)	11.2 (3.0)	11.8 (2.4)	11.3 (0.7)
length (mm)	b	7.2 (2.8)	9.7 (0.7)	7.8 (0.7)	8.5 (0.5)	8.8 (0.7)	9.2 (2.2)

The effect of kinetin on hypocotyl growth was nonsignificant, but the depression of root growth was highly significant at 10^{-4} M kinetin.

G. A. stimulated germination significantly only at concentration of 2.9×10^{-5} and 2.9×10^{-4} M ($P < .001$). This is in accord with the results of Kahn and coworkers (1956, 1957) and those of Lona (1956) for *Lactuca scariola*. There was no effect on hypocotyl growth or root length, which is in contrast to the findings of other authors where G. A. always significantly affected shoot growth (Brian, Hemming and Radley 1956).

For further experiments 10^{-5} M kinetin and 2.9×10^{-5} M G. A. were selected. The effect of R and FR radiation on their action was studied. The results for kinetin are summarized in Table III. It can be concluded that kinetin and R stimulate germination by different mechanisms whose effect is additive. FR reverses only the effect of R. This is in accord with Miller's findings (1956). It was again seen that kinetin significantly inhibited root growth. R and FR affect root growth, possibly due to their effect on the rate of germination. Their effect on root length is not changed by the presence of kinetin. Although in our own previous experiments (see Table I) kinetin did not significantly affect hypocotyl growth, in this set of experiments it caused a depression of growth of the hypocotyls. This depression is on the border of statistical significance.

TABLE III

The effect of R and FR on germination and growth in the presence of 10^{-5} M kinetin

Treatment	*% Germination*	*Root length (mm)*	*Hypocotyl length (mm)*
Water (Dark)	20.8	17.6	10.5
Water + R	44.2	20.7	10.5
Water + FR	14.8	17.2	9.5
Water + R + FR	12.0	13.0	7.8
Kinetin (Dark)	53.7	10.0	7.5
Kinetin + R	70.0	11.2	9.1
Kinetin + FR	44.6	8.1	6.0
Kinetin + R + FR	48.5	4.4	3.5

Statistical significance of the difference between treatment based on "t" test.

Treatments compared	P		
	Germination	*Root length*	*Hypocotyl length*
Water Dark/Water + R	—	—	Not significant
Kinetin Dark/Kinetin + R	< 0.001	0.001	Not significant
Water Dark/Water + FR	0.1—0.05	not. sign.	Not significant
Water Dark/Water + R + FR	Not significant	not. sign.	Not significant
Kinetin Dark/Water Dark	0.01	0.01	0.05—0.02
Kinetin + R/Water + R	0.001	0.05—0.02	Not significant
Kinetin Dark/Kinetin + FR	Not significant	not. sign.	Not significant
Kinetin Dark/Kinetin + R + FR	Not significant	not. sign.	0.1—0.05

A somewhat different picture is obtained for G. A. (Table IV). Germination was significantly stimulated by G. A. This stimulation was markedly reduced by FR. FR given immediately after R in the presence of G. A. only reversed the stimulation of R without reducing the germination below that of G. A. alone. This difference was caused either by the difference of time of application of FR in the two cases (see methods) or by the fact that the dose of FR was insufficient. To clarify this point, further experiments were designed. In one set of experiments FR was given 20 mins. after the seeds were put in water, and the usual R FR given after a further one hour and forty minutes. In a second set of experiments R was given as usual but the subsequent FR was doubled. The results with their controls are shown in Table V. "t" tests on these results showed that application of FR before and after R (FR+R+FR) did not significantly differ from R and FR (R+FR) in either water or G. A. However, a double dose of FR following R

TABLE IV

The effect of R and FR on germination in the presence of G. A. 2.9×10^{-5} M

Treatment	*% Germ.*	*S. D.*
Water (Dark)	12.0	6.3
Water + R	44.0	5.5
Water + FR	5.0	3.6
Water + R + FR	10.6	5.2
G. A. (Dark)	39.3	9.7
G. A. + R	66.4	18.5
G. A. + FR	24.8	7.4
G. A. + R + FR	35.0	4.9

Treatment compared	*P (based on "t" test)*
GA/GA + R	< 0.001
GA/GA + FR	< 0.001
GA + FR/GA + R + FR	< 0.001
GA/GA + R + FR	Not significant
Water/Water + FR	Not significant
Water+FR/Water+R+FR	Not significant

TABLE V

Effect of time of application and dose of FR on germination in G. A.

Treatment	% Germination Water	% Germination G. A. 2.9×10^{-5} M
None	18.4 (2.8)	41.3 (4.9)
FR	9.7 (3.5)	35.5 (5.1)
R	49.0 (5.6)	76.0 (5.3)
R + FR	14.0 (2.5)	41.0 (7.3)
FR + R + FR	13.3 (2.2)	42.0 (6.0)
R + 2FR	11.5 (5.9)	35.0 (9.1)

(R+2FR) significantly differed from R and FR in G. A. (P=0.02) but not in water (P=0.3).

It appears that if FR is given immediately after R then the dose determines whether only the R stimulation is reversed or also part of the G. A. stimulation. When the double FR dose is divided before and after R the same absolute dose of FR no longer affects the stimulation caused by G. A. This indicates that in addition to the absolute quantities of R and FR, the time sequence of their application affects the germination percentage obtained in G. A.

Lona (1956) concluded that the mechanism of stimulation of germination caused by R and G. A. may be different. In order to elucidate this point seeds were germinated in G. A. and given R after various length of time. The results are given in Figure 1. It is evident that this curve which represents the sensitivity of the seeds towards R as a function of time when germinated in G. A., is identical with that obtained in water (see Evenari and Neumann 1953). This observation together with the fact that part of the G. A. stimulation is reversed by FR and that the effects of R and G. A. are additive (Tables IV and V) makes the following supposition probable: The pathways along which R and G. A. act on germination is common for at least part of the germination process, i. e. that part which is reversed by FR. The mechanism of action of kinetin on the other hand seems to be completely different from that of R.

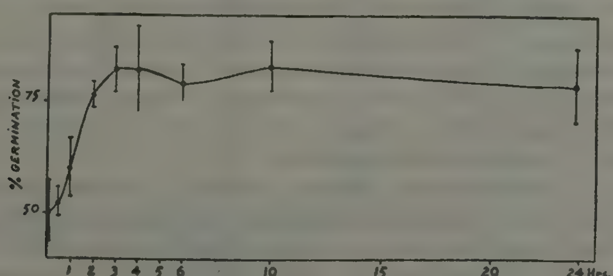

Figure 1

The change of sensitivity, with time, to red light of seeds germinated in G. A. Vertical lines indicate the standard deviation.

It was of interest to determine at what period of germination G. A. caused stimulation. As will be seen from Table VI, 3½ hrs. in G. A. already are sufficient to cause stimulation of germination. Moreover, when the seeds are kept in G. A. for 10 hrs. a maximum stimulation is obtained, the germination percentage being higher than that of seeds kept continuously (72 hrs.) in G. A. After more than 16 hrs, in G. A. maximal stimulation is no longer obtained. It appears, therefore, that G. A. affects the processes leading to germination during two different periods. The first period from 3—10 hrs. after the seeds were put in water and the second from 10 hrs. onwards.

TABLE VI

Germination % of seeds transferred after various times to or from G. A. (2.9×10^{-5} M)

Time (in hrs)	*% Germination To G. A.*	*From G. A.*
1	42 (9.3)	
1½		21 (5.1)
2	50 (6.0)	
3	49 (12)	
3½		38 (11.9)*
4	47 (13)	
5		46 (14.5)*
6	55 (11)	
7		55 (8.1)
10	28 (9.2)	78 (7.4)**
16		66 (11.6)***
24	41 (13.2)	70 (7.1)***
72 Water only		24 (7.3)
72 G. A. only		52 (10.1)

* Significantly different from water P=0.01.
** Significantly different from G. A. Control, P=0.01.
*** Significantly different from G. A. Control, P=0.05.
None of the transfers to G. A. differed significantly from the G. A. controls.

It may also be noted that G. A. stimulates germination even if applied 24 hrs. after placing the seeds in water.

Similar experiments with kinetin showed somewhat different results. The minimal time of application for full stimulation (70—80%) was 10 hrs. No optimal length of time of keeping the seeds in kinetin was noted. However, application of kinetin 24 hrs. after placing the seeds in water no longer caused maximal stimulation, germination being 40—50%. It appears therefore that for kinetin not the length but the time of application is of significance.

As G. A. did not significantly stimulate hypocotyl in the dark (Table II), it was decided to study this effect in more detail. The effect of G. A. on excised hypocotyls and roots was tested. No significant differences were observed in the dark. The effect of light on root and hypocotyl growth in G. A. was therefore tested on entire seedlings. Root growth was not affected by any of the G. A. concentrations test (2.9×10^{-8} — 2.9×10^{-4} M) in the light.

TABLE VII

Effect of G. A. on the length (mm) of excised hypocotyls in different continuous light conditions

	White light (100 f. c.)	*R* (0.5 f. c.)
Water	5.9**	11.0**
G. A. 2.9×10^{-5} M	14.6*	15.8*
G. A. 2.9×10^{-4} M	14.6*	—

Figures marked * are significantly different from their respective water controls ($P = 0.001$).
The figures for white and red light (marked **) differ significantly ($P < 0.001$) from each other.

The results obtained with hypocotyls are given in Table VII. G. A. significantly stimulates the growth of hypocotyls in light at concentrations of 2.9×10^{-5} and 2.9×10^{-8} M. White light is not essential nor were high intensities required as R alone at a very low intensity suffices to stimulate growth. The red filter used in these experiments was a Chance OR.2 filter transmitting from 600mμ onwards. Lower concentrations of G. A. than those given in Table VII were ineffective.

The next step was to test if R affected the growth of hypocotyls differently when applied at different times. For this purpose the seeds were irradiated with R continuously for 24 hrs. at different periods during their 72 hrs. germination. The results are given in Table VIII. It will be seen that all the treatments in G. A. result in increased hypocotyl growth as compared with water. The period at which R is given seems also to be of importance.

TABLE VIII

The influence of R Given continuously for 24 hrs at different times during a 72 hrs period on the length (mm) of hypocotyls. Concentration of G. A. was 2.9×10^{-5} M. D signifies dark period. Numbers in brackets give standard deviation

	1st 24 hrs.	*2nd 24 hrs.*	*3rd 24 hrs.*	*Hypocotyl length*	*P*
Water	R	D	D	6.7 (3.5)	0.001
G. A.	R	D	D	12.1 (5.0)	
Water	R	R	D	7.5 (3.6)	0.001
G. A.	R	R	D	12.2 (6.1)	
Water	R	R	R	8.2 (3.9)	0.02
G. A.	R	R	R	10.6 (5.4)	
Water	D	R	D	2.9 (2.9)	0.001
G. A.	D	R	D	8.2 (5.3)	
Water	D	D	R	4.9 (5.1)	0.02—0.01
G. A.	D	D	R	8.55 (7.0)	

Statistical significance of the difference between treatments based on *t* test.

G. A.	RDD/RRR	not signif.
	RDD/DRD	0.01—0.001
Water	RDD/RRD	not signif.
	RDD/RRR	not signif.
	RDD/DRD	0.01
	RDD/DDR	not signif.
	DRD/DDR	not signif.

REFERENCES

1. Brian, P. W. and Hemming, H. G., 1955, The effect of gibberellic acid on shoot growth of pea seedlings, *Phys. Plant., 8,* 669.
2. Brian, P. W., Hemming, H. G. and Radley, M., 1955, A physiological comparison of gibberellic acid with some auxins, *Physiol. Plant., 8,* 899.
3. Das, N. K., Patau, K. and Skoog, F., 1956, Initiation of mitosis and cell division by kinetin and IAA in excised tobacco pith tissue, *Physiol. Plant., 9,* 640.
4. Evenari, M. and Neumann, G., 1953, The germination of lettuce seed. III. The effect of light on germination, *Bull. Res. Counc. of Israel, 3,* 136.
5. Kahn, A., Goss, J. A. and Smith, D. E., 1956, Light and chemical effects on lettuce seed germination, *Plant. Phys., 31,* Suppl. XXXVII.
6. Kahn, A., Goss, J. A. and Smith, D. E., 1957, Effect of gibberellin on germination of lettuce seeds, *Science, 125,* 645.
7. Lang, A., 1956, Bolting and flowering in biennial *Hyoscyamus nigra* induced by gibberellin, *Plant. Phys., 31,* Suppl. XXXV.
8. Lona, F., 1956, Gibberellic acid induced germination of seeds and *Lactuca scariola* with dark inhibited peas, *Ateneo parmense, 27,* 641.
9. Marth, P. C., Audia, W. V. and Mitchell, J. W., 1956, Effect of gibberellic acid on growth and development of plants of various genera and species, *Bot. Gaz., 118,* 106.
10. Miller, C., 1956, A similarity of some kinetin and red light effects, *Plant. Phys., 31,* 318.
11. Wickson, J. M. and Thiman, K. V., 1956, The antagonism between kinetin and IAA in lateral bud development, *Plant. Phys., 31,* Suppl. XXVIII.

THE OCCURRENCE OF *CHLAMYDOMONAS* IN MASS CULTURE OF *CHLORELLA VULGARIS*

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ABSTRACT

Heavy infections of *Chlamydomonas* in *Chlorella* mass cultures are reported. These infections are shown to develop only when temperature conditions favour the infectant algae. The *Chlamydomonas* did not excrete substances toxic to *Chlorella*. It is concluded that infections can only occur if the temperature optima for growth of the infectant alga and *Chlorella* differ appreciably.

In previous papers (Mayer and Evenari 1955; Mayer, Eisenberg and Evenari 1956) it was reported that the mass culture methods used showed no infection by other organisms. In the present paper it is intended to report on the interference in mass culture of *Chlorella vulgaris* by *Chlamydomonas*.

METHODS

The mass culture was conducted in the tank previously described using the paddle-stirrer (Mayer, Eisenberg and Evenari 1956). The only changes were the following: The tank used was built of 4 mm sheet iron instead of concrete and the paddle-stirrer was operated directly by belt transmission to a motor, the shaft emerging from the tank through a waterproof gland. These changes caused smaller temperature lags and greater fluctuations than previously reported, but stirring was more accurately controlled.

The culture medium was as previously described. In some cases the ammonium sulphate was replaced by potassium nitrate, having an equivalent N content.

The growth in mass culture was determined as previously described.

Parallel to the mass culture, samples were removed from the tank to indoor cultures. These were conducted in constantly shaken Roux bottles, having an illumination of 400 f.c. and receiving a constant stream of air + 5% CO_2. The temperature of the indoor cultures was between 25—29°C. In mixed cultures the *Chlamydomonas* part of the population was estimated as follows:
The *Chlorella* cells were first counted. The mobile *Chlamydomonas* cells were then immobilized by adding a drop of chloroform to the culture and the total count determined. The *Chlamydomonas* was determined by difference. *Chlorella vulgaris*

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(Hopkins strain) was used throughout the experiments. This has a temperature optimum for growth of about 25—30°C (Burlew 1953). The *Chlamydomonas* infection was not further identified. It was easily differentiated from *Chlorella* by size and shape, being much bigger.

The experiments were conducted in the period January—May, 1957 in Jerusalem. This period was distinguished by an exceptionally cold winter with lower than normal temperatures. Heavy snow marked this winter. The temperatures of the outdoor tank were therefore very low, and variable.

RESULTS AND DISCUSSION

First observations were made in Jan.—Feb. 1957. The temperature of the tank normally reached 9°C at 9.00 in the morning. The *Chlorella* hardly showed any growth, reaching a maximum of 110 cells/mm^3. When the culture was a fortnight old, *Chlamydomonas* began to appear. The dry weight of the culture now rose but no steady yields could be obtained. The yields were almost entirely *Chlamydomonas*. The nutrient medium in this run contained KNO_3. After five weeks the tank was emptied and the experiment repeated. A run of one month was conducted. The results were entirely similar although the *Chlorella* reached a somewhat higher concentration — up to 600 cells/mm^3. During both runs, the *p*H of the culture was corrected by the addition of nitric acid, keeping it in the region of *p*H 6.5.

Shortly after the start of the run samples were removed from the tank and taken to the indoor cultures for comparison in growth. Under the conditions prevailing indoors, at a temperature of 27°C, the *Chlorella* grew rapidly, outstripping and later completely depressing the growth of the *Chlamydomonas*. Some typical comparison of indoor and outdoor growth is given in Table I. The outdoor tank yielded in this period a total yield of 103 g dry weight as compared with normal yields of 40—60 g per day. The indoor culture could be harvested daily, giving a yield of about 0.1 g/100 ml culture solution/day, half the culture being harvested.

These experiments were repeated, this time using the normal culture medium, containing ammonium sulphate. The following changes were also made. The outdoor culture was stirred day and night and given a continous CO_2 supply to bring it closer to the conditions of the indoor culture. The indoor cultures were given two treatments. Half were given the continuous air CO_2-stream as already described. The other half were given pure CO_2 for one hour per day as had been the practice in the mass culture tank. Throughout the experiments the *p*H of the cultures was controlled and kept both indoors and outdoors in the range 6.0—6.5. The mean midday temperature of the outdoor tank was 16—18°C, that of the indoor cultures 27°C. The results are summarized in Table II. It will be seen that giving continuous CO_2 and stirring continuously did not affect the ratio of *Chlamydomonas* to *Chlorella* in the outdoor culture. The motility of the *Chlamydomonas*

was somewhat affected, decreasing with the increasing CO_2 supply. This effect on motility was also observed in the indoor cultures.

In the indoor cultures the rate of growth of both organisms was determined by the amount of CO_2 given. The *Chlorella*, however, rapidly outgrew the *Chlamydomonas*, and the ratio was markedly in favour of *Chlorella*. The *Chlamydomonas* did, however, show some growth in contrast to the previous experiment (cf. Table I and II). The cause for this is not clear.

TABLE I

Growth of Chlorella and Chlamydomonas in indoor and outdoor cultures

Date	*Cell count/mm³ — outdoors*		*Cell count/mm³ — indoors*	
	Chlorella	*Chlamydomonas*	*Chlorella*	*Chlamydomonas*
27. II.57	60	—	—	—
3.III.57	510	250	—	—
11.III.57	700	250	700	250
12.III.57	610	400	2410	0
14.III.57	354	400	13200	0

TABLE II

Growth of Chlorella and Chlamydomonas in indoor and outdoor cultures under different treatments

	Outdoor			*Indoor*		
Date	*Treatment*	*Cell Count* *Chlorella*	*Chlamydomonas*	*Treatment*	*Cell Count/mm³* *Chlorella*	*Chlamydomonas*
17.IV.57	Normal	800	400		800	400
18.IV.57	Normal	600	240	continous CO_2 (1)	2660	400
				CO_2 further (2)	600	260
19.IV.57	Normal	650	300	(1)	8500	—
				(2)	650	320
21.IV.57	Normal	1000	250	(1)	13000	2000
				(2)	4500	250
23.IV.57	From 21.IV.57, continous stirring & CO_2	1200	400			
28.IV.57	Continous stirring & CO_2	1340	400			

Normal = stirring during daylight only and given CO_2 for one hour per day.

These results indicate that the temperature was the determining factor in the control of the ratio of *Chlorella* to *Chlamydomonas*. This was further borne out by a continuation of the experiments in June 1957. In this period *Chlamydomonas* was still present, the *Chlorella*, however, grew more rapidly and it was possible to obtain appreciable daily yields composed mainly of *Chlorella*, although lower than those obtained in previous years. In this period the midday tank temperature was 20°C or higher.

Despite this clear temperature effect it was deemed desirable to eliminate the possibility of the lack of growth of *Chlorella* being due to some excretion by the *Chlamydomonas*.

The infected culture medium from the tank was therefore treated in three different ways: centrifugation and Seitz filtration, centrifugation and autoclaving, and centrifugation only. Growth in these media was compared with that in freshly prepared medium all the samples being inoculated with a fresh sterile culture of *C. vulgaris*. The results are given in Table III.

TABLE III

Growth of C. vulgaris in culture medium taken from mass culture heavily infected with Chlamydomonas and given various treatments

	Tank medium			
	Centrifuged & autoclaved	*Centrifuged & sterilized by filtration*	*Centrifuged only*	*Control*
9.VI.57	1050	1850	1930	500
11.VI.57	600	6300	5530	3050
12.VI.57	10850	15150	19000	23600
13.VI.57	9450	13600	23350	20000
*p*H 13.VI.57	4.3	3.3	3.2	4.8
Final yield mg dry wt.	58.3	76.8	89.1	92.6
Observations			Small cells tending to clump	

From Table III it clearly emerges that there is no inhibition of growth of *Chlorella* by culture medium in which *Chlamydomonas* grow. It appears that the medium does contain some factor which on autoclaving decomposes and somewhat inhibits growth. The clumping obtained in untreated medium is presumably due to the non-sterility of this medium. This, resulting in some bacterial infection, is well known to cause clumping of *Chlorella* cultures.

It must be concluded from these experiments that under suitable conditions heavy infections by foreign algae are possible in *Chlorella* mass cultures. These infections

require suitable conditions, particularly of temperature. Apparently the infecting alga can only establish itself if its temperature optimum differs appreciably from that of *Chlorella*. Also the infectant algae must be present in adequate amounts in order to establish themselves rapidly.

At the same time it is worth noting that there is some indication that the growth of *Chlorella* is depressed by the infectant alga. Thus in Jan.—Feb. 1955 (Mayer, Eisenberg and Evenari 1956) maximum tank temperatures were recorded between 16 and 20°C and yields of 50 g were obtained from the tank daily. In these experiments in June 1957, when tank temperatures corresponded to those recorded in Jan.—Feb. 1955 yields did not exceed 30—35 g per tank per day.

This depression in growth was clearly not caused by some excretion caused by the infecting *Chlamydomonas* (Table III). At present we are unable to assign a definite cause to this depression. It is worth noting that *Chlamydomonas* is generally regarded as being somewhat difficult to culture and requiring media different from *Chlorella*. From the work reported here it was seen that under certain conditions it can grow rapidly and reach very high concentrations. Several hundred grams of dry *Chlamydomonas* were harvested during these experiments. The *Chlamydomonas* during drying produced a very noxious smell entirely different from that of *Chlorella*.

ACKNOWLEDGEMENT

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Note added in press:

The *Chlamydomonas* has been sent to Dr. R. Lewin, Woods Hole, Mass, for identification and has tentatively been described as *Chlamydomonas snowiae*.

REFERENCES

1. Burlew, J. S. Editor, 1953, Algal Culture from Laboratory to Pilot Plant, Carnegie Institute of Washington, Publication 600.
2. Mayer, A. M., Eisenberg, A. and Evenari, M., 1956, Studies on deep mass culture of algae in Israel, *Scientific Monthly, 83,* 198.
3. Mayer, A. M. and Evenari, M., 1955, The nutritional value of ovendried *Chlorella, Bull. Res. Counc. of Israel, 4,* 401.

THE EFFECT OF AUXIN AND ANTI-AUXIN ON GERMINATION OF LETTUCE SEEDS

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ABSTRACT

Certain IAA concentrations can stimulate the germination of dormant lettuce seeds. High IAA concentrations inhibited root growth. The anti-auxin PCIB did not affect the germination of lettuce seeds; it also did not affect the growth of lettuce roots.

The possibility that IAA * may play a part in the germination of seeds has been considered for many years. Although much experimental work was done on this subject, there is no agreement between the various results. Some of the results suggest an inhibition of germination by excess of auxins present in the seeds or fruits (Izard 1956, Naik 1954), while others report stimulation of germination by IAA (Hoffschlag 1948, Gerrard 1954). The controversy regarding the role of IAA in germination, therefore, still exists and no final conclusions can be reached (Audus 1953, Leopold 1955) Soeding and Wagner (1955) have suggested the idea that though IAA treatment does not affect the germination of non-dormant seeds, it nevertheless may stimulate dormant seeds. This did not prove to be right for Poa seeds. However, it was considered worthwhile to test this idea on lettuce seeds.

Seeds of the light sensitive variety Grand Rapids were used. The effect of IAA treatment was tried on two lots of seeds. One was purchased in 1954 from Ferry-Morse Seed Growers, California. The year of harvesting of this lot is not known, but is was apparently a few years earlier, as the California seeds were non-dormant, their dark germination being 60—80% at 26°C. The second lot was locally grown and harvested in 1954. The local seeds were very dormant and their germination under the same conditions was 4—10%.

The two lots of seeds were germinated in parallel, in various concentrations of sodium salt of IAA (*p*H 7.0) in the dark. The germination tests were carried out in Petri dishes, on filter paper and the percentage germination was calculated after 48 hours at 26°C. The experiments were repeated several times during two periods, June-July, 1955 and February, 1956. As there was no difference between

* The following abbreviations are used: IAA — Indolyl-3-acetic acid; PCIB — 4-chlorophenoxy-isobutyric acid.

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the results of these two periods, all the results were pooled and they are summarized in Figure 1.

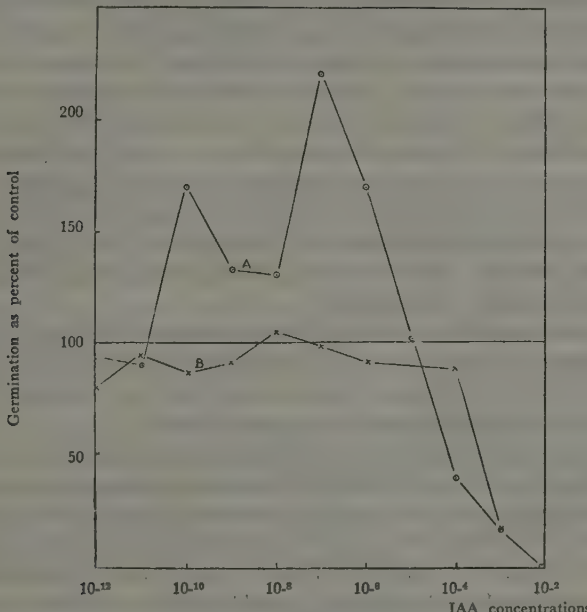

Figure 1
The germination of dormant (A) and non-dormant (B) lettuce seeds in various concentrations of IAA. The results are expressed as per cents of water controls. Mean water controls for lot A — 8%; mean water controls for lot B — 75%.

The significance of the effect of various concentrations of IAA on the percentage of germination of the two lots of seeds has been tested by the Quenouille's "point pair" test. In the dormant seed curve there are seven "point pairs", a number which would be reached or exceeded by chance only five times in 100 trials. The association of percentage germination with concentration of IAA is, therefore, statistically significant. It is of a curvilinear type — first either increasing or stable, later decreasing. This is not the case with the non-dormant seeds. In their curve there are only five "point pairs". The association of percentage of germination with IAA concentration is, therefore, not significant. The possible significance of the two peaks of curve A is not dealt with by this statistical treatment. As is seen from Figure 1, curve B is on the same level as the water controls, while curve A is on a much higher level. Evenari and Mayer (1954) also germinated the lettuce seeds in IAA (acid) solutions. They could not find any stimulation of germination. On the contrary, IAA proved to be an inhibitor. The

seeds used by Evenari and Mayer were not very dormant, their dark germination being of the order of 40%. Dormancy here refers to the whole lot of seeds. A dormant lot is one that contains a low percentage of seeds ready to germinate in optimal conditions of temperature and humidity, while a non-dormant lot contains a high percentage of such seeds. The "degree" of dormancy of every single seed is not considered here. It seems from the results of this paper that there is a certain stimulating effect of some IAA concentrations on the germination of very dormant lots of lettuce seeds. IAA does not affect the germination of non-dormant lots, except at concentrations higher that 10^{-5} M, when it always shows an inhibiting action. It may be mentioned here that neither the non-dormant nor the dormant seeds contain any apparent free IAA (Poljakoff et al, 1957). They do, however, contain several neutral growth promoting substances (Blumenthal-Goldschmidt — unpublished).

There is a remote possibility of the IAA being somehow linked with the activity of copper containing oxidative enzymes. This is suggested by the work of Ostrovskaya, (1956), who showed the stimulation of germination by IAA in rice seeds with a low copper content. No such effect existed in copper-rich seeds.

The occurrence of germination is determined by root emergence, which involves root growth. The effect of the various IAA concentrations on root growth in the two series of experiments was therefore also investigated. (Figure 2). The significance of the association of average root length (of seeds which germinated) with concentration of IAA has been tested by Tukey's "corner test" (Quenouille 1952). The score obtained is —11.5. A value of 11 would already be reached or exceeded by chance only five times in 100 trials. Therefore the association is statistically significant. It decreases first slowly and later steeply. Increasing concentrations of IAA inhibit, therefore, the growth of roots in dormant and non-dormant germinating lettuce seeds.

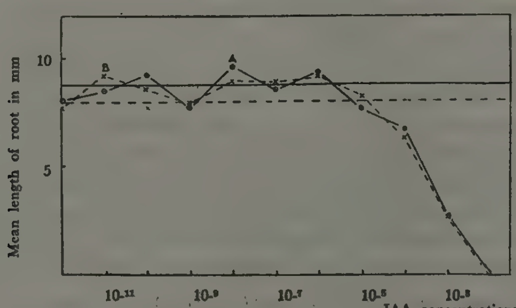

Figure 2
Mean root length, in millimeters, of dormant (A) and non-dormant (B) lettuce seeds germinated in various concentrations of IAA.

The effect of the anti-auxin PCIB on germination and root growth was investigated on the dormant lettuce seeds (Table I). The results of the PCIB treatments were compared to the water control with the aid of a "t" test. None of the differences is statistically significant. PCIB, therefore, does not affect germination of this lot of seeds. It also does not affect the growth of roots of lettuce seedlings grown at 20°C in the dark.

TABLE I

The effect of PCIB on germination and root growth of lettuce seeds

	Molar concentration of PCIB											
	None	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Percent germination	1.05 ± 5.7	9.8 ± 4.8	7.5 ± 4.5	8.5 ± 2.5	8.1 ± 3.5	4.2 ± 3.1	5.7 ± 2.3	6.9 ± 3.3	7.7 ± 5.3	7.8 ± 3.4	3.1 ± 1.8	1.4 ± 1.2
Root length in mm	5.7 ± 2.2	7.7 ± 1.6	6.9 ± 2.6	6.2 ± 2.2	6.1 ± 1.7	5.6 ± 1.0	6.3 ± 1.5	6.5 ± 1.8	5.9 ± 2.1	10.2 ± 2.2	5.0 ± 1.8	0.6 ± 0.6

In summarizing it may be said that some IAA concentrations can stimulate the germination of dormant lettuce seeds. None of the IAA concentrations used stimulated root growth. Inhibition of root growth was observed only at the higher IAA concentration. The anti-auxin PCIB does not affect either the germination of lettuce seeds or the growth of its roots.

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REFERENCES

1. Audus, L. J., 1953, *Plant Growth Substances,* L. Hill, London.
2. Evenari, M. and Mayer, A. M., 1954, The effect of auxin on the germination of lettuce seeds, *Bull. Res. Counc. of Israel, 4,* 81.
3. Gerrard, A., 1954, The effects of IAA on the germination and root growth of certain members of the Cruciferae, *New Phytol., 53,* 105.
4. Hoffschlag — As cited by Söding. See below.
5. Izard, C., 1956, Sur la germination des graines immature de certaines variétés de tabac, en présence de lumière et de sels d'urane, *Compt. Rend., 242,* 2027.
6. Leopold, A. C., 1955 *Auxins and Plant Growth,* Univ. Calif. Press, Berkeley and Los Angeles.
7. Ostrovskaya, L. K., 1956, Action of copper and heteroauxin on seed sprouting, *Fiziol. Rastenii, 3,* 73.
8. Naik, S., 1954, Effect of 1-naphthalenacetic acid on the rate of germination of seeds, *J. Indian Bot. Soc., 33,* 153.
9. Poljakoff-Mayber, A., Goldschmidt-Blumenthal, S. and Evenari, M., 1957, The growth substances content of germinating lettuce seeds, *Physiol. Plant, 10,* 14.
10. Quenouille, M. N., 1952, *Associated Measurements,* Butterworths Sci. Publ., London.
11. Soding, H. and Wagner, M., 1955, Mehrjährige Versuche über die Beeinflussung der Keimung und Entwicklung von *Poa annua* durch Behandlung der Früchte mit Wirkstoffen, *Planta, 45,* 557.

THE EFFECT OF EOSIN ON GERMINATION OF LETTUCE SEEDS

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ABSTRACT

The effect of eosin in the germination of lettuce was investigated. Eosin at high concentrations inhibits the germination of lettuce seeds. This is not due to a hypoauxinising effect. Eosin may act as a hypoauxiniser in Avena coleoptile growth. Eosin acts as a photosensitiser in the inhibitory effect of blue light on germination.

Eosin has been used for a long time as a sensitizer in some photochemical reactions (Fieser and Fieser 1950). It also has been reported as a photosensitizer in the photoinactivation of auxin (Skoog 1937). Other investigators consider eosin as a general "hypoauxinizing" agent, not necessarily in the presence of light (Lona and Bocchi 1952, 1955; Bocchi 1953 a, b).

Boas (1927) reported some peculiar effects of eosin on the geotropic response of roots. This effect may be interpreted as interference with normal auxin ratios. Bocchi (1953a) showed that eosin and indolyl acetic acid (IAA) can reverse each other's effect on germination in what she calls "hyperauxinized seeds". In a previous paper (Poljakoff-Mayber 1958) it has been shown that the germination of lettuce seeds during a certain period in their dormancy may be stimulated by IAA. It was considered worthwhile to investigate the effect of eosin on the germination of such "dormant" seeds, parallel to its effect on "nondormant" lettuce seeds, which are also insensitive to IAA.

Lettuce seeds of the variety Grand Rapids were used throughout the experiments. The IAA sensitive seeds were locally grown and harvested in 1954 ("local" seeds). The nonsensitive seeds were purchased in 1954 from the Ferry-Morse seed growers, California, U.S.A. ("imported" seeds).

The germination tests were carried out on filter paper in Petri dishes at 26°C in the dark. The germinating seeds were counted after 48 hours incubation and the percentage germination was calculated.

The effect af various concentrations of eosin on the germination of the two lots of seeds was investigated. The results are summarized in Table I. None of the differences are significant, except the inhibition of germination caused by the highest concentration of eosin. At all the other concentrations there is neither inhibition of germination of the dormant seeds nor stimulation of the non-dormant ones. Preliminary experiments for investigating a possible antagonism between IAA

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and eosin were conducted. Eosin at the concentrations of 1.44×10^{-6} M did not reverse the inhibition caused by 10^{-3} M IAA on germination of the non-dormant seeds. IAA 10^{-7} M, which stimulates the germination of dormant seeds, did not reverse the inhibition of germination of these seeds caused by eosin 1.44×10^{-3} M.

TABLE I

The effect of various concentrations of eosin on the germination of lettuce seeds

	Local seeds		Imported seeds	
Eosin conc.	*% Germination*	*Standard deviation*	*% Germination*	*Standard deviation*
0 (water control)	5.2	5.1	42.5	5.2
1.44×10^{-8} M	5.9	4.8	40.5	5.9
1.44×10^{-7} M	5.4	4.3	41.5	12.4
1.44×10^{-6} M	3.7	2.2	49.0	15.9
1.44×10^{-5} M	8.1	7.0	47.0	13.8
1.44×10^{-4} M	6.4	6.0	41.0	13.0
1.44×10^{-3} M	0.6*)	0.6	5.0*)	5.8

* = Significantly different from the respective water control ($P=0.001$).

TABLE II

The effect of various IAA and eosin concentrations on the extension growth of Avena coleoptile and Pea stem sections. These results are expressed as percent of initial length.

Eosin molar conc. / IAA mg/1	0	1.44×10^{-8}	1.44×10^{-7}	1.44×10^{-6}	1.44×10^{-5}	1.44×10^{-4}	1.44×10^{-3}
				Avena coleoptile sections			
0	121	122	122	125	113	113	104
1.0	152	131	139	136	128	116	107
2.5	146	149	136	136	131	114	104
5.0	142	153	147	136	132	115	106
				Pea internode sections			
0	107	111	106	111	111	111	108
1.0	136	128	132	137	136	110	104
2.5	144	129	123	122	132	116	104
5.0	126	130	112	122	119	118	107

No antagonistic effect of IAA and eosin could be demonstrated in the germination of lettuce seeds. Their combined effect on growth was therefore investigated. Avena coleoptiles and Pea internode sections were used in the conventional extension growth test using 5 mm long sections. The results are summarized in Table II. From these results it seems that eosin alone does not affect the growth of pea stem

sections but inhibits the growth of Avena coleoptiles. The inhibitory concentrations are from 1.44×10^{-5} M and above. Linser (1955) reports inhibition of growth of Avena coleoptiles by eosin concentration of 10^{-4}%, which is equivalent to 1.44×10^{-6} M. It must however be kept in mind that Linser used a completely different growth test which may possibly explain the difference. It follows from Table II that high eosin concentrations (1.44×10^{-4} and 1.44×10^{-3} M) markedly reverse the growth stimulation caused by IAA in both growth tests used. Therefore, eosin may be considered to certain extent as an IAA antagonist or "hypoauxinizing agent". But this antagonism is not apparent in the IAA induced germination mechanism of lettuce seeds.

The germination of the Grand Rapids seeds is controlled by some photochemical reaction sensitive to Red and Far Red radiation (Borthwick et al. 1952, Evenari et al. 1953), while blue light was considered neutral. Recently Evenari et al. (1957) proved its effectiveness in inhibiting and stimulating germination of lettuce seeds. Eosin's absorption spectrum is in the blue-green region. Therefore its effect as a photosensitizer of the effect of blue light on germination of lettuce seeds was investigated. The local "dormant" lettuce seeds were germinated in water and eosin in the usual way, in the dark, but after one hour of incubation they were illuminated for 5 minutes with blue light and returned to darkness. The blue light was obtained by filtering white light of 500 f. c. at the seed level through a filter. The filter used was Corning Glass No. 5113 violet filter. Its transmission is bettween 350 and 480 mμ and above 2000 mμ with a peak at 400—420 mμ. The results are summarized in Table III. Within each series only the highest eosin concentration differed significantly from the water control, thus confirming again the results of Table I. Between the two series, concentration of 1.44×10^{-3} M and 1.44×10^{-4} M eosin in blue light, significantly inhibited germination as compared with those without blue illumination, the significance being at the level of 1.0 percent. The effect of eosin of 1.44×10^{-5} M and blue light was only at the level of 5 percent significance.

TABLE III

The effect of eosin and blue light on the germination of local lettuce seeds

	Eosin only % *Germination*	*S. D.*	*Eosin and blue light* % *Germination*	*S. D.*
0	15.7	4.6	9.8	4.1
1.44×10^{-8}	22.8	10.0	7.6	7.0
1.44×10^{-7}	20.4	9.9	8.1	4.0
1.44×10^{-6}	10.1	8.0	7.4	6.2
1.44×10^{-5}	19.6 ***	6.7	11.2 ***	7.2
1.44×10^{-4}	27.9 **	13.0	9.1 **	4.4
1.44×10^{-3}	8.0 *,**	3.0	1.8 *,**	1.0

* Significantly different from the respective water controls (P=0.01).
** Significantly different between the series (P=0.01).
*** Significantly different between the series (P=0.05).

In conclusion it may be said that eosin does not affect the germination of lettuce seeds except at very high concentration when its effect is inhibitory. IAA does not reverse this inhibiting effect of eosin. A reversal of IAA stimulation by eosin does exist in the effect on straight growth of coleoptile and pea stem sections. Eosin may also serve as a photosensitizer in inhibition of germination of lettuce seeds by blue light.

REFERENCES

1. Boas, F., 1927, *Ber. dtsch. bot. Ges., 45,* 61.
2. Bocchi, A., 1953a, *Nuovo G. bot. ital., 60,* 1.
3. Bocchi, A., 1953b, *Ateneo parmense, 24,* 1.
4. Borthwick, H. A., Hendricks, S. B., Parker, M. W., Tool, E. H. and Tool, V., 1952, *Proc. Nat. Acad. Sci.,* Wash., *38,* 662.
5. Evenari, M. and Stein, G., 1953, *Experientia, 9,* 94.
6. Evenari, M., Neumann, G. and Stein, G., 1957, *Nature London,* (in press).
7. Fieser, L. F., and Fieser, M., 1944, *Organic Chemistry,* D. C. Heath and Company, Boston.
8. Linser, H., 1955, *Z. Pfl. Ernähr. Düng., 69,* 215.
9. Lona, F. and Bocchi, H., 1952, *Nuovo G. bot. ital., 59,* 511.
10. Lona, F. and Bocchi, H., 1955, *Beitr. Biol. Pfl., 31,* 332.
11. Poljakoff-Mayber, A., 1958, *Bull. Res. Counc. of Israel, 6D,* 8.
12. Skoog, F., 1935, *J. cell. comp. Physiol., 7,* 227.

SOME FURTHER STUDIES ON THE DIRECT OXIDATION OF GLUCOSE IN GERMINATING LETTUCE SEED

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ABSTRACT

Glucose 6-phosphate and 6-phospho gluconic acid dehydrogenases were shown to be present at a high level in dry seeds and decreased in activity with time of germination. A shifting metabolic pathway is suggested. Neotetrazolium chloride from various sources showed different sensitivity to the malic dehydrogenase system.

In a previous paper (Mayer et al. 1957) it was suggested that a direct glucose oxidative mechanism might function in germinating lettuce seeds. In the present study the changes in glucose 6 phosphate (G6-P) and 6 phosphogluconic acid (6-PG) dehydrogenases were followed during germination. The neotetrazolium reduction method of Glock and Jensen (1953) was used throughout the experiments. The determinations were made in seed or seedling homogenates, which were prepared as previously described (Mayer et al. 1957).

During the investigation it was found that various sources of neotetrazolium chloride showed different response to the dehydrogenase action. Neotetrazolium chloride from various firms was therefore compared for sensitivity as hydrogen acceptors for malic dehydrogenase in the presence of DPN. The results are summarized in Table I. Due to the very large differences in the sensitivity of the indicator dye, neotetrazolium from one firm only, Light, was used throughout the experiments.

TABLE I

The activity and solubility of neotetrazolium chloride from different commercial firms. Activity determined as reduction by the malic dehydrogenase system from lettuce seedlings.

Commercial firm	Description of salt	Solubility	Activity as γ neotetrazolium chloride reduced
1. Light and Co. Ltd.	Light yellow powder	v. soluble in cold water	64
2. T. Gurr Ltd.	Light yellow powder	v. soluble in cold water	57
3. Bios Lab. Inc.	Greyish-brown powder	soluble in hot water with residue	46
4. N.B.Co. (1st sample)	Brownish-yellow powder	soluble in hot water with residue	39
5. N.B.Co. (2nd sample)	Dark brown powder	sparingly soluble in hot water. Large residue	37

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The change in G.6P and 6.P.G. dehydrogenase activity with increasing length of germination time is given in Table II. The results are expressed as γ neotetrazolium chloride reduced during 3 hours of incubation at 26°C.

A consideration of Table II shows that there is no statistically significant rise of dehydrogenase activity with increasing time of germination. G6-P and 6-PG dehydrogenase have approximately the same activity. Both show higher activity in dry seeds, than either malic or succinic dehydrogenases (Mayer et al. 1957).

TABLE II

Change in dehydrogenase activity with increasing time of germination. Results expressed as Neotetrazolium chloride reduced.

Length of Germination	Enzyme activity 6-PG	G 6-P
Dry seeds	31.0±6.0	25.3±5.9
24 hours	26.0±5.8	22.0±3.6
48 hours	38.0±13.0	41.6±4.1
72 hours	45.0±21.0	33.4±7.6
96 hours	20.0±7.4	27.6±5.5

The final concentrations were:

Substrate	.013
TPN	.0033%
Dye	.13%

Total volume 4.5 ml. *p*H 7.3.
None of the differences are statistically significant.

This suggests that the direct oxidative pathway of glucose may be of particular significance in the germination process proper and early stages of growth. It has been previously shown that sucrose is the first substrate oxidised during this period (Poljakoff-Mayber 1952). In the later stages of seedling growth the glycolytic and Krebs cycle mechanism develop rapidly (Mayer et al. 1957, Poljakoff-Mayber 1955).

As the number of cells of every seedling increases with growth, the lack of change in the activity of the two dehydrogenases studied actually indicates a decrease in their activity per cell. Consequently, a change in oxidative pathways during development suggests itself.

REFERENCES

1. Mayer, A. M., Poljakoff-Mayber, A. and Appleman, W., 1957, *Physiol. Plant.*, *10*, 1.
2. Glock, E. and Jensen, C. O., 1953, *J. Biol. Chem.*, *201*, 271.
3. Poljakoff-Mayber, A., 1952, *Pal. J. Bot. Jerusalem Ser.*, *5*, 180.
4. Poljakoff-Mayber, A., 1955, *J. exp. Bot.*, *6*, 313.

THE JOINT ACTION OF THIOUREA AND COPPER ON THE GERMINATION AND GROWTH OF LETTUCE

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ABSTRACT

A germination stimulation by copper chloride given for short periods of time is reported. This effect appears to be different and independent from the stimulation caused by thiourea.

In a previous paper the physiological action of thiourea on germination and growth was discussed (Poljakoff-Mayber et al. 1958). As it is known that thiourea acts as a copper chelating agent it is possible that copper modifies the physiological action of thiourea.

It was intended to test whether copper could reverse the growth inhibition and or the germination stimulation of lettuce caused by thiourea.

The effect of copper alone on the germination and growth of lettuce is not known. To clarify this, the effect of copper chloride on these processes was studied. Lettuce seeds, variety Grand Rapids, were germinated as previously described (Poljakoff-Mayber et al. 1958), in various concentrations of copper chloride. The results are summarized in Table I. It will be seen that high concentrations of copper inhibit germination and growth while low concentrations have no effect. Although it appears that the concentrations 10^{-4}, 10^{-5} M copper show an optimum effect on germination and hypocotyl growth, the differences from the controls were found to be statistically non-significant.

TABLE I

Effect of $CuCl_2$ on the germination and growth of lettuce after 72 hours germination at 26°C in the dark.

Copper conc. M	*Mean % germination*	*Mean root length*	*Mean hypocotyl length*
0	51.6	12.7	7.2
10^{-8}	44.0	11.2	5.2
10^{-7}	46.6	12.0	5.7
10^{-6}	51.0	11.53	5.7
10^{-5}	55.8	12.15	7.65
10^{-4}	58.0	6.2	7.0
10^{-3}	51.6	1.4	2.1
10^{-2}	0	0	0
10^{-1}	0	0	0

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As copper and thiourea react in solution, giving a precipitate, it was impossible to apply them together to the seeds. It was therefore attempted to place the seeds in solutions of copper and to transfer them after various time intervals to thiourea, and vice versa. The length of time of application of thiourea needed to give optimal germination was determined. It was found that seeds transferred after 24 hours from thiourea 5×10^{-2} M to water, gave maximal germination. This was about 90% as compared with 40% for water controls and 70—80% for shorter or longer periods in thiourea.

Seeds were therefore germinated for 24 hours in thiourea and then transferred, in blue light, to various concentrations of copper chloride and incubated for a further 48 hours at 26°C. Germination and growth were then determined. Copper chloride concentrations 10^{-2}, 10^{-3}, 10^{-4} M did not modify the effect of thiourea on growth or germination in any way.

As it was possible that the copper was at once precipitated at the seed surface by the thiourea already present, the procedure was reserved. Seeds were germinated for 24 hours in copper chloride of the above concentrations and then transferred for a further 48 hours to thiourea. Again the copper chloride did not change the effect of thiourea. However, seeds transferred after 24 hours from copper chloride to water appeared to germinate to a higher percentage than water controls, especially at 10^{-2} M $CuCl_2$ (Water control 57%, $CuCl_2$ 10^{-2} M 84%). The effect on growth was as in Table I. It was therefore decided to investigate whether $CuCl_2$ could indeed stimulate germination, and what was the optimal time of application.

Seeds were transferred from 10^{-2} M $CuCl_2$ to water after various time intervals during the first twenty-four hours of germination. The results showing the germination percentage after 72 hours are given in Table II.

TABLE II

% germination of lettuce seeds transferred from $CuCl_2 10^{-2}$ M to water after various lengths of time. Germination carried out at 26°C in the dark.

Time in $CuCl_2$	*% Germination at 72 hrs.*
0	56
15 min	45
30 min	34
60 min	55
90 min	50
2 hrs	69
2½ hrs	50
3 hrs	53

From Table II it will be seen that two hours in $CuCl_2$ 10^{-2} M cause a stimulation of germination. This stimulation is significant at the 2% level as compared with water controls. It will be seen therefore that the same copper concentration can

stimulate or inhibit germination, depending on the length of treatment with $CuCl_2$.

It can be inferred Cu^{++} ions have some function in the germination process. Thiourea can chelate Cu^{++} ions and is present in appreciable amounts in seeds treated with it (Mayer 1956).

The failure of Cu^{++} ions to reverse the action of thiourea on germination and growth suggests that the mechanisms of action of the two substances are not directly related. Therefore the stimulation of germination caused by Cu^{++} ions and by thiourea is presumably also due to separate mechanisms.

It was previously suggested (Mayer et al. 1957) that the metabolic pathways in germinating seeds are very flexible and given to change by different treatments. It seems possible therefore that the results here obtained can be explained on a basis of shifting metabolic pathways. It could be suggested that Cu^{++} ions and thiourea each can divert the metabolism of the seeds along different paths, to the same final result — germination.

This hypothesis is being further investigated.

REFERENCES

1. Poljakoff-Mayber, A., Mayer, A. M. and Sachs, S., 1958. *Ann. Bot.* (in press).
2. Mayer, A. M., 1956, *J. exp. Bot.*, *7*, 93.
3. Mayer, A. M., Poljakoff-Mayber, A. and Appleman, W., 1957, *Physiol. Plant.*, *10*, 1.

SOME FURTHER INVESTIGATIONS ON THE GROWTH ACTIVE SUBSTANCES EXTRACTABLE FROM DRY LETTUCE SEEDS

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ABSTRACT

Dry lettuce seeds contain a variety of growth active substances. These substances have different solubility properties. Alcohol, water and alcohol-water mixtures were used as extraction media. Different solvents extracted substances of different properties. Water-alcohol mixtures extracted more substances than any of the other media.

It is not certain whether the substances extracted in water or water-alcohol were in their natural form or whether they were liberated enzymatically or otherwise during the extraction.

Dry lettuce seeds contain no free IAA, but it is possible that they contain a precussor which liberated IAA during the extraction in water.

In a previous paper (Poljakoff-Mayber et al. 1957) two acidic growth inhibitors were reported from alcoholic extracts of dry lettuce seeds. Wareing and Foda (1956) also reported growth inhibitors extracted from lettuce seeds. The location of these two inhibitors on the chromatogram was very similar in this work and that of Wareing. The extraction methods were different however. Wareing and Foda (1957) used water extracts of material previously extracted with ether while we used alcoholic extracts. It was decided, therefore, to try to compare several methods of extraction in parallel and to investigate the effect of the extractable substances on germination of lettuce seeds and on the growth of their roots.

MATERIAL AND METHODS

Lettuce seeds, variety Grand Rapids (G. R.) were used. The seeds were purchased from Ferry-Morse Seed Growers, California, in 1954. 25 g lots of dry seeds were used in every one of the three different methods of extraction reported below.

(a) The seeds were put into boiling absolute ethanol, ground in a mortar and extracted at —8°C for twenty-four hours.

(b) The seeds were put into boiling absolute ethanol and an equal amount of water added. The seeds were ground in a mortar and extracted at 4°C for twelve hours.

(c) The dry seeds were ground in a mortar at room temperature and extracted in cold water at 4°C for two hours.

After the extraction was finished, the liquids were separated from the solids by means of centrifugation. The solids were washed twice with the solvent used for extraction. The solutions were collected and evaporated under reduced pressure to an aqueous syrup.

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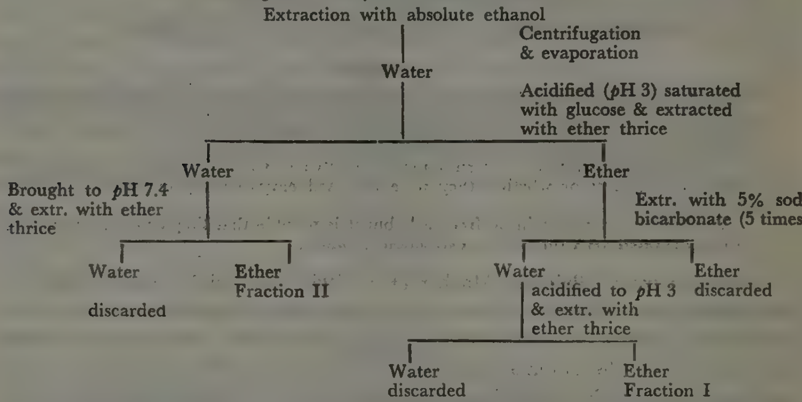
Dry peroxide-free ether was used in the following treatments.

The three extracts obtained were treated as described in the following schemes (Figures 1 and 2).

Figure 1

The procedure of the extraction method a

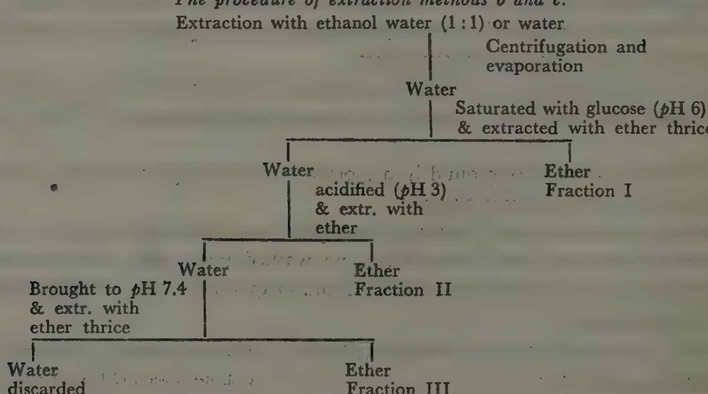


A – Extract (a) (Figure 1) was further treated as previously described by Poljakoff-Mayber et al. (1957). The resulting acidic fraction is designated as Fraction I. The water residue of the first ether extract was neutralized with N/1 NaOH to pH 7.4 and extracted three times with ether. The combined ether extract is designated as Fraction II.

B – Extracts (b) and (c) were treated as follows (Figure 2):

Figure 2

The procedure of extraction methods b and c.



I. The original extracts (*p*H 6) were saturated with glucose and extracted three times with ether. These ether extracts were Fractions I (b) and I (c).

II. The aqueous residue of the above extracts was acidified with concentrated phosphoric acid to *p*H 2.8—3.0, saturated with glucose and extracted with ether, giving Fractions II (b) and II (c).

III. The aqueous residue, after extracting Fractions II, was neutralized with N/1 NaOH to *p*H. 7.4 and extracted three times with ether, giving Fractions III (b) and III (c).

The final ether extracts were reduced in volume to 0.5—1 ml and put on Whatman No. 1 paper strips. The strips were 10 cm wide and 57 cm long. The extracts were applied to the paper on a line 9.5 cm long, 7 cm from the edge. Every fraction was quantitatively divided between two strips.

The chromatograms were run in isopropanol-ammonia-water (10:1:1) for 20 hours, using the descending method. After the solvent had run 45—50 cm the chromatograms were dried and every one of them was divided longitudinally into two strips. Thus, every strip represented 6.25 g initial material. These strips were cut horizontally according to their fluorescence in U. V. light and the biological activity of the different areas of the chromatograms was tested in the following biological assay methods.

1. Avena coleoptile extension test, as described previously (Poljakoff-Mayber et al. 1957).

2. Lettuce seed germination test. Seeds of the variety G. R. were sown in the dark for 24 hours at 26°C. Ungerminated seeds were taken out and peeled, this being done at 26°C and in inert blue light. 25 such peeled seeds were put into each of the Petri dishes containing the parts of the chromatograms tested. The dishes were put into dark for another 24 hours. Then the germinated seeds were counted and the percent germination calculated.

This procedure enabled us to eliminate the fluctuations of germination percentage in the dark. All the normally dark germinating seeds, completed their germination in 24 hours. Germinability of the seeds which did not germinate during the 24 hours period, was increased by peeling (Evenari and Neumann 1952). The resulting percent of germination of the controls after further 24 hours was then very constant — around 50%.

3. Lettuce root growth. The length of the roots of the above mentioned germinated seeds were measured and the mean length was calculated, taking into account only the number of the seeds that did germinate.

EXPERIMENTAL RESULTS

Acid Fractions — Table I

Fraction I from method 'a' of extraction and Fraction II from method 'b' of extraction yielded three inhibitors af Rf's 0.05—0.15, 0.40—0.60, and 0.90.

TABLE I

The biological activity of acid substances extracted from dry lettuce seeds by various methods of extraction

Rf	0.05—0.15			0.10—0.30			0.40—0.60			0.70—0.85 Y			0.90		
Method of extraction	C	R	G	C	R	G	C	R	G	C	R	G	C	R	G
Alcohol (Fraction I)	I	I	I	—	—	—	I	None	I	—	—	—	I	None	I?
Alcohol water (Fraction IIb)	I	I	I	P	I	None	I	None	I	P	I	I	I	None	I?
Water (Fraction IIc)	—	—	—	P	I	I	I	I	—	—	—	—	—	—	—

C — Coleoptile extension growth test
R — Root growth test
G — Germination test

I — Inhibition
— — The zone did not show any activity
None — No effect in this special test
P — Promotor

TABLE II

The biological activity of the neutral substances extracted from dry lettuce seeds by various methods of extraction

Rf	0.05—0.20			0.20			0.40			0.45—0.50			0.55—0.80			0.85			0.80—1.00		
Method of extraction	C	R	C	C	R	G	C	R	G	C	R	G	C	R	G	C	R	G	C	R	G
Alcohol (Fraction II)	P	I	I	I	I	I	—	—	—	—	—	—	None	P	I	—	—	—	I	I	I
Alcohol water (Fraction IIIb)	P	None	None	I	I	I	I	I	None	P	I	I	P	I	I	None	I	I	—	—	—
Water (Fraction IIIc)	P	I	None	I	I	I	—	—	—	P	P	None	I	I	I	None	I	I	—	—	—

C — Coleoptile extension growth test
R — Root growth test
G — Germination test

I — Inhibition
— — The zone did not show any activity
None — No effect in this special test
P — Promotor

The inhibitor at Rf 0.05—0.15 is a general inhibitor, inhibiting coleoptile extension, root growth and germination. The inhibitor at Rf 0.40—0.60 inhibits coleoptile extension and germination but not root growth. Inhibitor at Rf 0.90 inhibits mainly coleoptile extension but sometimes it also inhibits germination. Its effect on germination was inconsistent in repeated extractions. The (b) method of extraction in Fraction II yielded also two growth promoters, at Rf's 0.15—0.30 and 0.70—0.85. Both these substances promote the extension growth of coleoptiles but inhibit root growth and germination. The first one is a weak inhibitor but the second is a strong one. Fraction II (c) yielded a general inhibitor at an Rf 0.40—0.60 and a growth promoting substance at the Rf of 0.10—0.30. This growth promoting substance promotes the coleoptile extension growth but inhibits root growth and germination.

Initial Fractions

The initial Fractions I (b) and I (c) extracted at *p*H 6.0 yielded very similiar results to those of the acid fractions.

Neutral Fractions II and III — (Table II)

Fraction II from method 'a' of extraction yielded two general inhibitors, at Rf 0.20 and 0.80—1.00, as well as a growth promoter at Rf 0.05—0.20. This last substance inhibits germination and root growth. A fourth substance at Rf 0.55—0.80 does not affect coleoptile extension growth but inhibits germination and promotes root growth. Fraction III (b) yielded one general inhibitor at an Rf of 0.20 and another inhibitor at an Rf of 0.40. This inhibitor inhibits root growth but does not inhibit germination. A third substance at an Rf of 0.85 is a root growth and germination inhibitor but does not affect coleoptile sections. In the same fraction three promoters were also present: one at Rf 0.10, with no effect on roots and germination; the other two at Rf 0.50 and 0.70—0.80 which inhibit root growth and germination.

Fraction III (c) yielded very similar results to III (b) as it is evident from Table II.

Wareing and Foda (1956) report that their zone "A" showed a bright green fluorescence. In extracts prepared by method (b) we found a light green zone which gave a bright green fluorescence. This zone was found at an Rf 0.06 in chromatographs of the extract syrup which was not subjected to any further treatment. It was not found in the final ethereal extract of the same extraction method nor in any other way of extraction. It had no biological activity in any of the tests used. The substances of this zone are apparently not identical with the fluorescent substance found by Wareing and Foda.

DISCUSSION

Dry lettuce seeds contain several growth active substances which behave differently under different methods of extraction. It is evident from Tables I and II that the alcohol-water mixture extracts many more substances than each of the components alone. Some substances which are not soluble in either of the solvents alone dissolve in their mixture.

The fact that the alcohol-water extract contains more substances than each solvent alone could effect the migration of the substances on the chromatogram. For instance, zone 0.45—0.50 of Fraction III (b) (Table II) could be a result of the interference of two substances; one from zone 0.55—0.80 in Fraction II, and the other of zone 0.40—0.50 of Fraction III (c).

Substances which appear in similar zones on the chromatograms of different extracts and which show different activity could nevertheless be identical. This may be due to different solubility in the various extraction media which results in different concentration, and consequently different biological activity. Such an example may be found in fraction II (b) and II (c) at the Rf 0.10—0.30 (Table I) where there is inhibition of germination by the water extract which does not exist in the alcohol water fraction. Another example is the zone at Rf 0.55—0.80 (Table II). In Fraction III (b) there is growth promotion of coleoptiles, in Fraction III (c) growth inhibition and no effect in Fraction II. This substance may be a genuine water soluble auxin. At low concentrations it promotes and at higher concentrations inhibits growth. At very low concentrations, which correspond to its alcohol solubility, it does not effect coleoptile growth, promotes root growth but still inhibits germination.

It is uncertain whether all these substances are extracted by the various media in their natural form. They may be liberated enzymatically or otherwise during extraction with water or water-alcohol. Thus the active substance in zone Rf 0.85 in Fraction III (b) and III (c), (Table II), may be formed either from the substances in zone 0.55—0.80 or 0.80—1.0 in Fraction II, or from a completely different precursor, which does not appear in the chromatograms. This problem is now being further investigated.

There is no doubt that each zone on the chromatogram could contain more than one substance. This could cause natural interference in biological activity. Attempts are now being made to effect better separation of the substances on the chromatograms.

Various workers found a number of growth active substances in the different plant materials investigated. Many of these substances correspond to ours in the place of their appearance on the chromatogram and in their biological activity. Thus Lexander (1953) found in wheat roots an active substance at an Rf 0.16 which promotes root and coleoptile growth. This substance may be similar to that

found here at an Rf of 0.10—0.30 (Table I). It may also correspond to that which Nitsch (1955) found at Rf 0.23 and postulated to be indole pyruvic acid. No substance similar to the α accelerator (Kefford 1955) in the acidic fraction was found. In the neutral fraction a substance at an Rf 0.05—0.20 occurred which may be considered a genuine auxin, as it promotes coleoptile growth and inhibits root growth.

Wareing and Foda (1956) found a growth inhibitor in extracts prepared at *p*H 6 at an Rf 0.10—0.30. In this work an acidic growth promoting substance was found at the same Rf (Table I), which was also extractable at *p*H 6. The inhibitor found there had a lower Rf — 0.05—0.15. The other inhibitor found by Wareing and Foda (1956) was at an Rf 0.40—0.50. An acidic inhibitor of a very similar Rf is described in this paper. Kefford (1955, 1955a) and Teubner (1953) also found an inhibitor at a very similar Rf. This inhibitor may therefore be of a more general occurrence and interest in plant material.

The neutral substance at Rf 0.85 could be indolyl aceto nitrile, (IAN) but it could not be identified by a colour reaction.

As IAN is extractable to some extent in the acid fraction, the mixture appearing at Rf 0.7—0.85 might also be IAN.

In a previous paper (Poljakoff-Mayber et al. 1957) it was concluded that the dry seeds contain no free indolyl acetic acid (IAA). In the present work a substance was found at an Rf 0.10—0.30, which could be IAA. This substance appears only in the water or alcohol-water extracts. It never appeared in absolute alcohol extraction. It could not be identified by a colour reaction. It is reasonable, therefore, to think that if this substance is really IAA, it is liberated during the extraction from some bound form.

ACKNOWLEDGEMENTS

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REFERENCES

1. Evenari, M. and Neumann, G., 1952, The Germination of lettuce seeds. II. The influence of fruit coat, seed coat and endosperm upon germination, *Bull. Res. Counc. of Israel, 2,* 75.
2. Kefford, N. P., 1955, The growth substances separated from plant extracts by chromatography, *J. exp. Bot., 6,* 129.
3. Kefford, N. P., 1955a, The growth substances separated from plant extracts by chromatography. II. The coleoptile and root elongation properties of the growth substances in plant extracts, *J. exp. Bot., 6,* 245.
4. Lexander, K., 1953, Growth regulating substances in roots of wheat, *Phys. Plant., 6,* 406.
5. Nitsch, J. P., 1955, Free Auxins and free tryptophane in the strawberry, *Plant Physiol., 30,* 33.

6. Poljakoff-Mayber, A., Goldschmidt-Blumenthal, S. and Evenari, M., 1957, The growth substances content of germinating lettuce seeds, *Phys. Plant.*, *10*, 14.
7. Taubner, F. G., 1953, Identification of the Auxin present in apple endosperm, *Science*, *118*, 418.
8. Wareing, P. F. and Foda, H. A., 1956, The possible role of growth inhibitors in the dormancy of seeds of xanthium and lettuce, *Nature Lond.*, *178*, 908.
9. Wareing, P. F. and Foda, H. A., 1957, Growth inhibitors and dormancy in xanthium seeds, *Phys. Plant.*, *10*, 266.

EFFECT OF RED LIGHT AND GIBBERELLIC ACID ON THE TEMPERATURE-INHIBITED GERMINATION OF LETTUCE SEEDS

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ABSTRACT

Heat dormant seeds are insensitive to red irradiation, if this is given at the high temperature. The irradiation is effective if following the light treatment the seeds are transferred to a lower temperature. The presence of gibberellic acid during heat treatment prevents the development of full heat dormancy. The possible mechanisms of red light and gibberellic acid effects are discussed.

It has been known for some time that treatment of plants with the gibberellins can produce effects, some of which are similar to the effects produced by red light i. e., mainly flowering and germination (Borthwick et al. 1954, Lona 1956, Kahn et al. 1956, Evenari et al. 1958). Lately there was a report on the "replacement" by gibberellins of the secondary light requirement, in a case of temperature-induced dormancy, of light non-sensitive seeds (Kahn, Goss and Smith 1957). It was considered worth while to investigate the effect of gibberellic acid on the temperature induced dormancy of light sensitive lettuce seeds.

Lettuce seeds, variety Grand Rapids, were used throughout the experiments. The germination tests were carried out in Petri dishes on filter paper at 30°C and 26°C in the dark. The seeds used in these experiments germinate to 30—40 percent at 26°C while at 30°C their germination was usually less than 5%.

The effect of red light (R) on the germination at 30°C was investigated. Seeds were germinated in water at 30°C for 48 hours, then some of the dishes were illuminated for 30 seconds with red light. The others were kept in the dark. Half of the R stimulated and half of the dark plates were transferred to 26°C, while the other half were kept at 30°C. The germinating seeds were counted in all of them after further 48 hours and 96 hours of incubation. The results are summarized in Table I.

TABLE I

The effect of red light (R) in reversing the temperature induced germination inhibition

Treatment	*Germination percent*	
	—R	*+R*
48 hrs. 30°C, then 48 hrs. 26°C	4.0	12.4
48 hrs. 30°C, then 48 hrs. 30°C	3.0	3.8
48 hrs. 30°C, then 96 hrs. 26°C	8.4	11.5
48 hrs. 30°C, then 96 hrs. 30°C	4.2	4.0
96 hrs. at 26°C	30.0	96.0

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As it is evident from these results, if after illumination the seeds were transferred to 26°C, there was a slight reversal of the inhibition of germination caused by the high temperature. If, however, after illumination the seeds were returned to 30°C, there was not any light effect at all. From the results of Table I, it also seems that in the case of seeds kept in the dark and transferred to 26°C the temperature inhibition is slowly abolished with time. To clarify this point, seeds were germinated for 48 hours at 30°C and then transferred to 26°C. The germination percentage was counted after 2, 4, 6 and 8 days. There was no increase in germination percent with the increased time in 26°C. The germination percent in all these cases varied between zero and 8%.

It was thought that 30 seconds of R may be too short a period in which to antagonize the prolonged temperature effect. The temperature treatment was therefore shortened, while the light treatment was prolonged. The results are summarized in Table II. As can be seen from Table II, the dormancy induced by two days at 30°C is much too deep to be reversed even by 10 minutes R, when the seeds are kept in 30°C continuously. If, however, after illumination the seeds are transferred to a lower temperature, the light is effective in reversing the inhibiting influence, 30 seconds or 10 minutes are equally effective in reversing the temperature effect. Two hours at 30°C were not sufficient to induce heat dormancy if the seeds are transferred at the end of the two hours to 26°C. R treatment in such a case does not differ from the ordinary light treatment under normal conditions. If, however, the heat treatment at 30°C is continued after the two hours then dormancy is induced. This heat induced dormancy is broken by light treatment given after two hours. Here the dose of light was of importance, 10 minutes being more effective than 30 seconds.

TABLE II

Germination percent of temperature inhibited lettuce seeds after various treatments of temperature and light.

Treatment	*Germination percent*		
	– R	*+ R 30 sec.*	*+ R 10 min.*
48 hrs. 30°C, then 48 hrs. 26°C	1.5	12.0	12.0
48 hrs. 30°C, then 48 hrs. 30°C	0.5	3.0	0.0
2 hrs. 30°C, then 48 hrs. 30°C	3.5	29.0	50.0
2 hrs. 30°C, then 48 hrs. 26°C	26.0	91.0	90.0

R – red light treatment

The effect of gibberellic acid (G. A.) on the temperature inhibition of germination was investigated. The seeds were germinated in various concentrations of G. A. for four days, either continuously at 30°C or with a transfer to 26°C. The results are summarized in Table III. As it is apparent from these results, only the highest

concentration of G. A. is effective in abolishing the inhibitory effect of the high temperature. It seems that at 26°C it is more effective than at 30°C, but this difference is statistically not significant. This high concentration of G. A. has a true effect in reversing the temperature induced dormancy of the seeds. Such a reversal coult not be achieved by R treatment that in itself is sufficient for the stimulation of germination under normal conditions.

TABLE III

The effect of gibberellic acid on the temperature inhibited germination of lettuce seeds (Figures in brackets give the standard deviation)

Temperature treatment	*G. A. conc. in mg/l*	*Germination percent* 0.0	0.01	0.1	1.0	10.0	100.0
48 hrs. 30°C, then 48 hrs. 26°C		2.0	5.0	5.0	2.5	5.0	44.0 (12.1)
96 hrs. 30°C		0.5	—	—	1.0	2.0	20.0 (16.1)

The combined effect of R. and G. A. on the germination was investigated. The results are summarized in Table IV. As it is apparent from these results, G. A. of the concentration of 100 mg/l always reverses the temperature induced inhibition. The action of G. A. and R is much more pronounced when the seeds are transferred to 26°C than when they are kept throughout at 30°C.

TABLE IV

The combined effect of red light and gibberellic acid on the temperature inhibited germination of lettuce seeds (Figures in brackets give the standard deviation)

Serial No.	*Temperature and light treatment* / *G. A. conc. in mg/l*	*Germination percent* 0.0	0.01	0.1	1.0	10.0	100.0
I	48 hrs. 30°C → R 30 sec. → 48 hrs. 26°C	17.1	13.0	18.5	17.1	30.9	86.0 (3.9)
II	48 hrs. 30°C → R 30 sec. → 48 hrs. 30°C	3.9	2.0	2.0	5.0	8.1	50.5 (16.0)
III	48 hrs. 30°C → 48 hrs. 26°C	0.5	—	—	2.2	7.3	52.9 (35.2)
IV	96 hrs. 30°C	2.0	—	—	4.3	7.6	33.0 (10.6)

All these results may be summarized as follows : Seeds made fully heat dormant by high temperature treatment are insensitive to R when it is given at the same high temperature. This heat dormancy could be broken to some extent by R if the seeds are transferred after the light treatment to a lower temperature. Lowering the temperature alone, without light treatment, has no effect on germination.

If, however, the seeds are treated with R before developing full heat dormancy (2 hours at 30°C) continued heat treatment does not completely prevent germination.

The presence of G. A. during the heat treatment prevents the development of full heat dormancy. This effect of G. A. is not affected by temperature change.

When G. A. and R treatments are combined and then the temperature is lowered, the effect of both treatments is additive.

These results point to the possibility that high temperature treatment either brings to accumulation in the seeds of some inhibiting substance, or causes destruction of some compound vital to germination. The longer the seeds are kept in the high temperature the more of the inhibitor is accumulated or stimulator destroyed. The temperature effect may exert its influence merely by changing the relative rate of various reactions which are normally proceeding in the imbibed seeds. These changes produce the heat dormancy. The reaction initiated by R, whatever it may be, is temperature sensitive. If R is applied early during the heat treatment full heat dormancy cannot develop.

As for the G. A. treatment, it was already suggested in an earlier paper (Evenari et al. 1958) that a part of the pathway along which G. A. and R lead to germination, is different in each of them. This is borne out by results of this paper.

It may be concluded, therefore, that the "replacement" by G. A. of the light requirement of seeds is actually a bypass of some block in the metabolism, which is achieved by a mechanism different from the one released by R treatment. The two mechanisms lead to a point whence there is a common metabolic path, eventually leading to germination.

REFERENCES

1. Borthwick, H. A., Hendricks, S. B., Toole, E. H. and Toole, U. K., 1954, *Bot. Gaz., 115,* 205.
2. Evenari, M., Neumann, G., Goldschmidt-Blumenthal, S., Mayer A. M., and Poljakoff-Mayber, A., 1958, *Bull. Res. Counc. of Israel, 6D,* 65.
3. Lona, F., 1956, *L'Anteneo Parmense, 27,* 641.
4. Kahn, A., Goss, J. A. and Smith, D. E., 1956, *Suppl. Plant. Physiol, 31,* 37.
5. Kahn, A., Goss, J. A. and Smith, D. E., 1957, *Science, 125,* 695.

THE INTERACTION OF THIOUREA AND ASCORBIC ACID IN THEIR EFFECT ON GERMINATION AND GROWTH

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ABSTRACT

It thas been shown that there is an interaction between ascorbic acid and thiourea in their effect on germination and growth. The effect on germination is synergistic, that on growth is antagonistic. The results are discussed in the light of the known properties of thiourea and ascorbic acid. It is suggested that the antagonism on growth may be due to the inhibition by thiourea of the ascorbic acid oxidase and consequent depression of the amount of dehydro-ascorbic acid in the seeds. The effect on germination is a specific one which cannot be explained on the basis of the known functions of ascorbic acid in plant tissues.

In a previous paper the effect of thiourea on germination and growth of lettuce was reported (Poljakoff-Mayber, Mayer and Zacks, 1958). Ascorbic acid has been shown to affect the germination inhibition caused by coumarin and 2.4-D, (Levari, Mayer and Evenari 1952). In addition, it has been shown that thiourea inhibits the ascorbic acid oxidase of germinating lettuce (Mayer 1958). It seemed likely therefore that the ascorbic acid content of thiourea treated seeds could be higher than that of untreated seeds. The effect of thiourea might therefore be due to the presence of ascorbic acid as such in the seeds. If this is correct, then externally added ascorbic acid should modify the effect of thiourea on germination and growth. This seems even more likely, as ascorbic acid has already been shown to modify the effect of germination inhibitors.

The effect of thiourea on the germination and growth of lettuce in the presence and absence of ascorbic acid was therefore investigated.

METHODS

Locally grown lettuce seeds, Variety Grand Rapids, were used throughout the experiments. They were germinated at 26°C in the dark. Germination and growth were determined as previously described (Poljakoff-Mayber, Mayer and Zacks 1958). Ascorbic acid, in its free form, was freshly prepared every two to three days and kept in the cold at 2°C.

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RESULTS

Lettuce seeds were germinated for 72 hours in three different concentrations of thiourea in the presence and absence of ascorbic acid. At the end of the 72 hours, germination and growth of hypocotyl and roots was determined. The results are summarized in Table I. Regression lines were fitted to the data of Table I according to the equation

$$y = a + bx \tag{1}$$

These are given in Figures 1—3. The variability of the results is given in Table II.

As will be seen from Table I and Figure 1, ascorbic acid alone does not significantly affect germination. It does, however, markedly increase the stimulation caused by thiourea.

TABLE I

The effect of thiourea and ascorbic acid on the germination and growth of lettuce. Concentration of ascorbic acid was 75 mg%

	Thiourea conc. M	*Thiourea only*	*Thiourea + ascorbic acid*
Germination %	0	17.8	21.2
	1×10^{-2}	26.8	44.3
	2.5×10^{-2}	32.0	47.5
	5×10^{-2}	50.5	76.5
Hypocotyl length (mm)	0	11.1	4.9
	1×10^{-2}	6.8	4.3
	2.5×10^{-2}	3.9	3.9
	5×10^{-2}	2.1	1.8
Root length (mm)	0	11.2	5.8
	1×10^{-2}	8.1	5.2
	2.5×10^{-2}	4.7	3.2
	5×10^{-2}	1.9	1.8

TABLE II

Data on the standard errors of a & b, SE (a) & SE (b), and the variance around the regression lines $S^2\ y/x$ for Figures 1, 2, 3 in the equation $y=a+bx$.

	SE (a)	*SE (b)*	$S^2 y/x$
Germination without ascorbic acid	± 0.120	± 0.158	0.0098
with ascorbic acid	± 0.112	± 0.163	0.0095
Root length without ascorbic acid	± 0.817	± 2.279	0.00085
with ascorbic acid	± 0.982	± 4.692	0.0415
Hypocotyl length without ascorbic acid	± 0.266	± 1.932	0.056
with ascorbic acid	± 0.396	± 1.405	0.0075

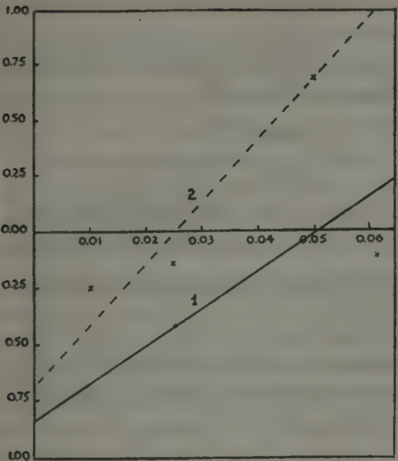

Figure 1
Regression lines for germination data
$y \equiv$ Normit % of germination,
$x \equiv$ Thiourea concentration.
1) ——— Thiourea $y = -0.84 + 16.50\ x$
2) - - - - - Thiourea + ascorbic acid
$y = -0.69 + 27.34\ x$

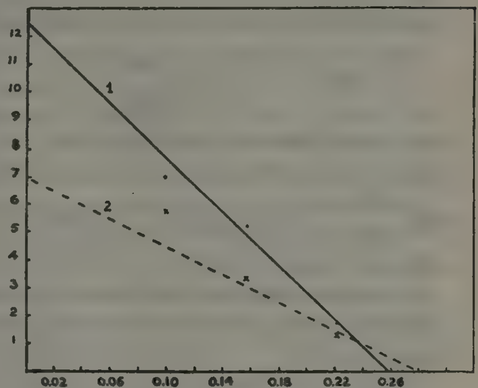

Figure 2
Regression lines for root length data
$y \equiv$ Mean root length $x \equiv$ Thiourea conc.
1) ——— Thiourea $y = 12.50 - 48.09 \sqrt{x}$
2) - - - - - Thiourea + ascorbic acid
$y = 6.93 - 24.72 \sqrt{x}$

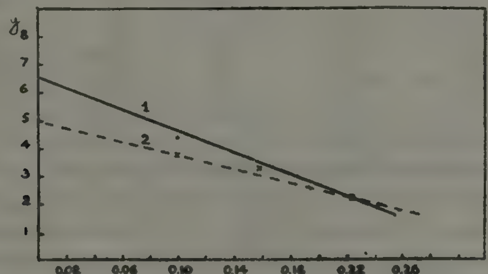

Figure 3
Regression lines for hypocotyl length
$y \equiv$ Mean hypocotyl length $x \equiv$ Thiourea conc.
1) ——— Thiourea $y = 6{\cdot}52 - 19.33 \sqrt{x}$
2) - - - - - Thiourea + ascorbic acid $y = 5.00 - 12.26 \sqrt{x}$

From Figure 2 and Table I it is evident that ascorbic acid inhibits root growth. The roots treated with thiourea and ascorbic acid were shorter then those treated by either of them alone. Their effect is not additive however. The difference in slope of the regression line (Figure 2) clearly points to an interaction between the two compounds. A very similar picture emerges for hypocotyl growth (Table I

and Figure 3) although the absolute difference between treatments were smaller. Here too the regression lines point to an interaction.

It was possible that the effect of ascorbic acid was due to its strong reducing action. Thiourea exists in two taumoteric forms as in equation (2).

$$\text{(2)} \qquad SC\begin{matrix} \diagup NH_2 \\ \diagdown NH_2 \end{matrix} \rightleftharpoons HSC\begin{matrix} /\!\!/ NH \\ \diagdown NH_2 \end{matrix}$$

which may differ in biological activity. The addition on a reducing agent like acsorbic acid produces a shift in the equilibrium of (2) to the right. If the form $HSC\begin{matrix} /\!\!/ NH \\ \diagdown NH_2 \end{matrix}$ is the active one, than germination stimulation would increase on the addition of ascorbic acid.

To test this possibility, the effect of a reducing agent, cysteine hydrochloride and an oxidizing agent, dehydro-ascorbic acid, on germination in the presence of thiourea was investigated.

TABLE III

Effect of thiourea in the presence and absence of dehydro-ascorbic acid 75 mg % and cysteine hydrochloride 75 mg % on germination.

	% Germination					
Thiourea conc.	*Thiourea only*		*Thiourea and dehydro-ascorbic acid*		*Thiourea and cysteine hydrochloride*	
		SD		*SD*		*SD*
0	13.4	7.7	8	1.7	11.0	1.0
1.0	27.0	9.3	30	5.7	23.0	2.9
2.5	41.0	11.9	37	7.7	38.5	10.9
5.0	59.0	13.5	50	8.7	51	7.0

The results are shown in Table III. It will be seen that cysteine hydrochloride in no way resembled ascorbic acid in its action. Dehydro-ascorbic acid did not antagonise the action of thiourea in any way. Neither substance alone affected germination. It can be concluded therefore that the ascorbic acid effect is not due merely to its reducing action.

DISCUSSION

From the results described it will be seen that there is a clear interaction between thiourea and ascorbic acid. This interaction is one of synergism in the case of germination and of antagonism in the case of growth. The dosage response curves (see Figures 1, 2, 3) show a linear dependence on the square root of the dose for growth while for germination the dose itself is linearily related to the Normit percentage. These two facts together clearly point to completely different mechanisms of action of thiourea on germination and growth. Thiourea has been shown to inhibit ascordic acid oxidase (Mayer 1958). Such an inhibition must lead to a rise

in the ratio ascorbic acid/dehydro-ascorbic acid in the seeds. If this is in any way related to germination stimulation by thiourea than the addition of ascorbic acid to the seeds should stimulate germination while addition of dehydro-ascorbic acid should inhibit it. It has been shown that this is not the case. Hence thiourea does not act on germination because it changes the ascorbic dehydro-ascorbic acid ratio.

The picture, however, is somewhat different for growth. Marre and his collaborators, (Tonzig and Marre 1955, Marre and Arrigoni 1957) have shown that dehydro-ascorbic acid inhibits growth. In the experiments reported here, the addition of ascorbic acid to whole seeds had a similar effect. This may be due to the conversion of ascorbic acid to dehydro-ascorbic acid in the seeds. This is entirely feasible in view of the high ascorbic acid oxidase activity present in the seeds (Mayer 1958). Thiourea inhibits the oxidase and hence the conversion of ascorbic acid to dehydro-ascorbic acid. As a result thiourea in part abolishes the inhibition caused by ascorbic acid and consequently the apparent antagonism in the effect on growth of a thiourea and ascorbic acid appears.

During germination the interaction of thiourea and ascorbic acid has been shown to be a specific one and not due to the reducing properties of the latter. Kamson-Rappaport (1958) has shown that lettuce seeds contain small amounts of ascorbic acid. The ascorbic acid content rises, as germination and growth proceed but is not related to the germination percentage. The only known function of ascorbic acid in plant tissue is that it is part of an electron transport system. From the results reported here it appears that ascorbic acid has a function in some additional process during germination. When germination proceeds normally, only very small amounts of ascorbic acid are required for this additional process. If however the electron transport system is disturbed by thiourea (Mayer 1958) the second process, in which ascorbic acid participated, increases in importance. As a result the addition of ascorbic acid to the seeds treated with thiourea increases the germination stimulation of the latter. The nature of the second process in which ascorbic acid participates is at present entirely problematical.

ACKNOWLEDGEMENT

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REFERENCES

1. Kamson-Rappaport, M., 1958, Changes in ascorbic acid content of germinating lettuce seed, *Bull. Res. Counc. of Israel, 6D,* 126.
2. Levari, R., Mayer, A. M. and Evenari, M., 1952, The effect of various metabolites on the action of some germination inhibitors, *Bull. Res. Counc. of Israel, 2,* 27.
3. Marre, E. and Arrigoni, O., 1957, Metabolic reactions to auxin I, the effect of auxin and glutathine and the effects of glutathine on growth of isolated plant parts, *Physiol. Plant., 10,* 289.
4. Mayer, A. M., 1958, Ascorbic acid oxidase in germinating lettuce seed and its inhibition, *Physiol. Plant.* (in press).
5. Poljakoff-Mayber, A., Mayer, A. M. and Zacks, S., 1958, Interaction of thiourea and IAA in their action on germination and growth of lettuce, *Ann. Bot.* (in press).
6. Tonzig and Marre, E., 1955, The auxin-ascorbic acid oxidase interaction as related to physiological activity of auxin, *Rend. Inst. Lombard Sci. and Let., 89,* 243.

THE RESPIRATION OF LETTUCE SEEDS GERMINATED AT A HIGH TEMPERATURE

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ABSTRACT

Lettuce seeds germinated at high temperatures show a higher respiration rate during the imbibition period than those germinated at lower temperatures. As the high temperature prevents germination the respiration rate declines steadily after the 22nd hour of imbibition.

Light sensitive lettuce seeds, for full germination in the dark, require temperatures of 18—20°C or below. At higher temperatures the germination percentage is much lower and decreases with increased temperatures. (Evenari 1952, Evenari, Neumann and Stein 1953). Rise in temperature, within biological limits, has long been known to increase respiration rate in general. (James 1953). For seeds this is known from investigations with stored wheat grains (Bailey and Jurgar 1918). Similar results are reported by Crocker and Barton (1953). It was of interest, therefore, to investigate the respiration of lettuce seeds at temperatures stimulating respiration but inhibiting germination.

Lettuce seeds, variety Grand Rapids, were used throughout the experiments. The respiration was measured by Warburg's "Direct Method" (Umbreit et al. 1947). 100 mg amounts of seeds were put in the main compartment with 0.25 ml of water, either with or without KOH in the centre well. The flasks, wrapped in black plastic sheets, were attached to the manometers and shaken in the constant temperature bath. The readings were made at one hour intervals. The experiments were run at two temperatures, 26°C and 30°C. At the end of the experiment the germinated seeds were counted and the percentage of germination calculated. While at 26°C the germination percentage varied between 25% and 30%, at 30°C it varied between 0 and 3%.

The oxygen consumption, the CO_2 evolution and the changes in RQ are summarized in Figure 1. The curves of Figure 1 do not give the actual readings but the moving average calculated for every four successive readings. The gas exchange i sexpressed as μl/hour/100 mg initial weight of seeds.

After four hours in water the seeds are already almost fully imbibed. During this time at 26°C the respiration increased from below 5μl/100mg/hr in dry seeds, to about 35μl/100mg/hr. There was no appreciable change in CO_2 evolution

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until the fourteenth hour, when the actual germination process was already under way and the rootlets became visible. Oxygen consumption showed a decrease in its rate just prior to this period, between the 10th and the 14th hour, after the seeds were put in water. From the 14th hour and on the respiration increased steadily until the 20th hour, when all the seeds capable of germination in the dark showed rootlet emergence.

The results of these measurements correspond with the results reported in a previous paper (Evenari et al. 1955).

In the experiments carried out at 30°C, the initial rise in respiration during imbibition was higher than at 26°C. This was specially marked in the CO_2 evolution. The steep initial rise in the respiration curve was followed by a continuous slight increase at the rate of respiration. There was no such sudden increase as was observed at the 14th hour in the seeds kept at 26°C. The seeds at 30°C, as already stated, did not germinate. The respiration rate tended to decrease after the 22nd hour. This was much more pronounced in the CO_2 evolution than in oxygen consumption. Similar phenomena of decrease in respiration rate for seeds of Amaranthus, Impatiens and Rumex kept in temperatures unfavourable for germination are reported by Barton (1945). In all these cases the seeds were kept in the unfavourable conditions for a much longer period than in the experiments presented here.

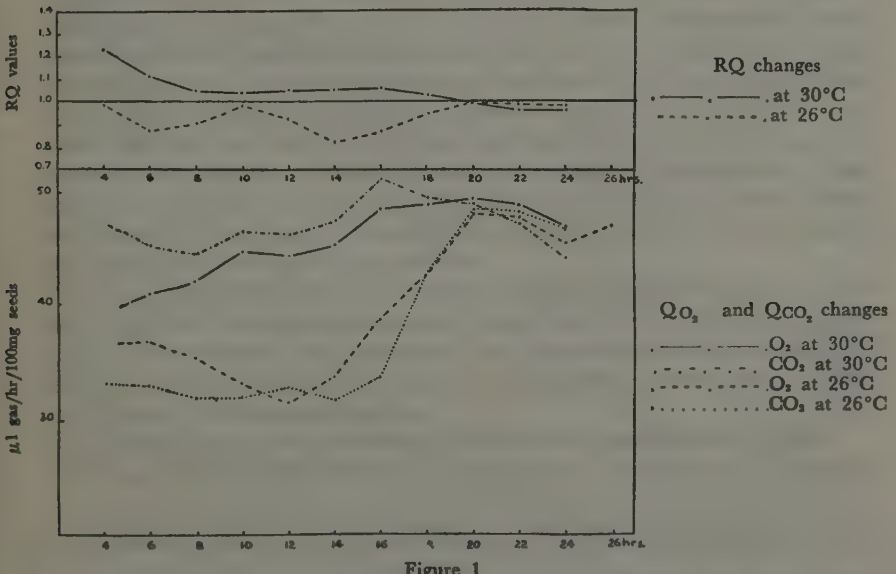

Figure 1

The changes in Q_{O_2} Q_{CO_2} and RQ during germination of the lettuce seeds at 26° and 30°C.

An increase in temperature usually causes also an increase in the RQ (James 1953). There is an agreement between this and the present results. In the very first hours the RQ of seeds kept at 30°C is considerably higher than unity. Already at the 8th hour RQ approaches unity and remains around it. But most of the time the RQ of the seeds kept at 30°C is higher than that of the seeds kept at 26°C.

There is no possibility to draw any conclusion from the results presented here, regarding the nature of the metabolic changes induced by the high temperature. These changes, as indicated by the respiratory behaviour of the seeds, are of vital importance, as they block the process usually leading to germination.

REFERENCES

1. Bailey, C. M. and Jurjar, A. M., 1918, Respiration of stored wheat, *Journ. Agr. Res.*, *12*, 685.
2. Barton, L. V., 1945, Respiration and germination. Studies of seeds in moist storage, *Ann. N. Y. Acad. Sci.*, *46*, 185.
3. Crocker, W. and Barton, L. V., 1953, *Physiology of Seeds*, Chronica Botanica, U.S.A.
4. Evenari, M., 1952, The germination of lettuce seed. I. Light, temperature and coumarin as germination factors, *Pal. Journ. Botany, Jer. Ser.*, *5*, 138.
5. Evenari, M., Neumann, G. and Stein, G., 1953, Factors modifying the influence of light on germination, *Nature*, *172*, 452.
6. Evenari, M., Neumann, G. and Klein, S., 1955, The influence of red and infrared light on the respiration of photoblastic seeds, *Physiol. Plant.*, *8*, 33.
7. James, W. O., 1953, *Plant Respiration*. The Clarendon Press, Oxford.
8. Umbreit, W. W., Burris, R. H. and Stanffer, J. F., 1947, *Manometric Technique*, Burgess Publishing Co., Minneapolis.

THE MECHANISM OF GERMINATION STIMULATION BY ALTERNATING TEMPERATURES

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ABSTRACT

1. Studies were made on the effect of short rapid changes in temperature on dark germination of light-sensitive lettuce seeds.
2. No correlation was found with the rate of change, or the duration of the change; a very good correlation was found with the temperature change itself as measured in the dishes, at temperatures between 23°C to 32°C.
3. The sequential reactions hypothesis is rejected; an organised structure is indicated as the site of action, and two suggestions as to its nature are discussed.

A method for the breaking of dormancy in seeds, buds, spores and insect eggs by the use of alternating temperatures has been known and applied (Crocker and Barton 1953, Morinaga 1926, Samish 1954, Sussman 1956). It has been suggested that this effect is mechanical in nature (Crocker and Barton 1953, Morinaga 1926).

Johnson et al. (1954) applied their *absolute rate theory* to the effect of fluctuating temperatures on sequential reactions; a change in the steady state conditions of temperature will change the rate of the reactions and the concentrations of the metabolites in the sequence differently than will the effect of some other steady state condition at a different temperature. Toole et al. (1955) in their discussion of the effect of temperature changes and their interaction with light on germination, suggested that the effect of alternating temperatures is of such a type. They suggested that dormancy may be the result of a subcritical concentration of some metabolite, the momentary rise of which, at the time of the temperature change, may bring it to the level of "breaking" the dormancy. This mechanism can be correct only under the following conditions:

(a) The effect should depend on the rate of the temperature change; a very slow change will not differ from successive steady-states of temperature conditions.

(b) At the effective rate there should be a dependence on the period during which the material is kept at the alternate temperature, as some time is needed for a change of concentration to occur.

(c) Changes in opposite directions should lead to results similarly opposed.

To check this hypothesis of changed reaction rates, the effect of a temperature

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change on lettuce seed germination was studied by analysing the effect of the rate of change, the duration of the temperature treatment and the amount of change on germination. Only the conditions stated under (a) and (b) have been fully tested and will be reported here.

METHODS AND MATERIALS

Lettuce seeds of the light-sensitive variety, Grand Rapids, were germinated in Petri dishes in distilled water on filter paper. The dishes were kept in light-tight tins in a thermostat at 22°C. Percent germination was determined after 48 hours; the results are the averages of 4 dishes. Temperature readings were made by a copper constantan thermocouple that touched the wet filter paper in one of the dishes in one tin. The readings were considered representative for all the dishes in the tins.

The temperature changes were achieved by transferring the tins from 22°C to thermostats of various higher temperatures, 30°C to 100°C for different periods, and back to 22°C.

RESULTS

Petri dishes were transferred from 22°C to various temperatures for various periods of time, as described in Methods and then returned to 22°C.

The change of temperature after return to 22°C was determined after being kept for various periods of time in the alternate temperatures. From this, a value was obtained showing the maximal temperature attained after return to 22°C, and being kept for various periods of time at the alternate temperatures. These maximal temperatures were then plotted against the time in the other temperature. The temperatures used were 30°, 37°, 45°, 60°, and 80°C. The results are shown in Figure 1.

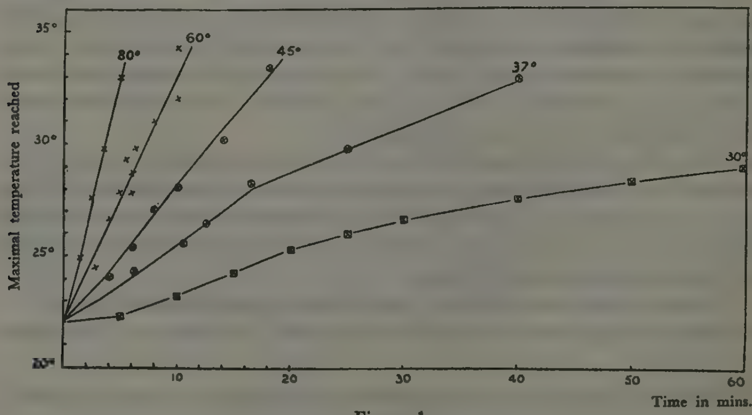
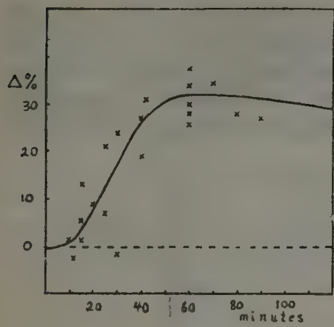


Figure 1
The change in the maximum temperature in the dishes with time in the different thermostats. The transfer is from 22°C and back again.



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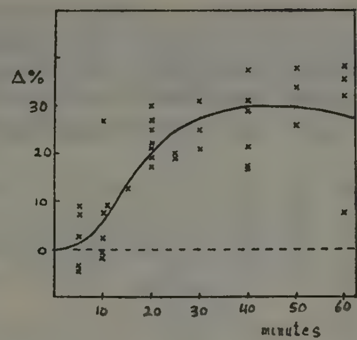

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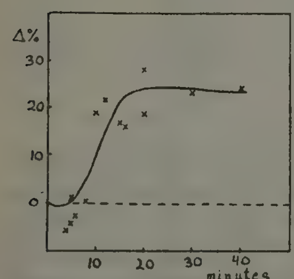

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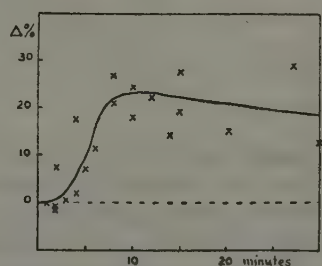

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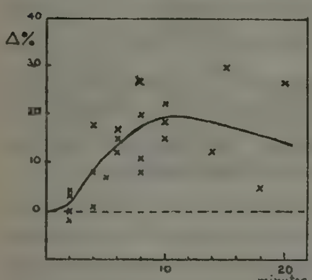

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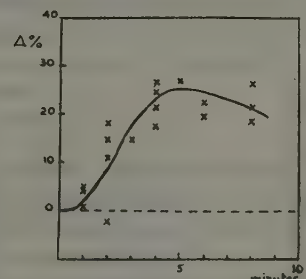

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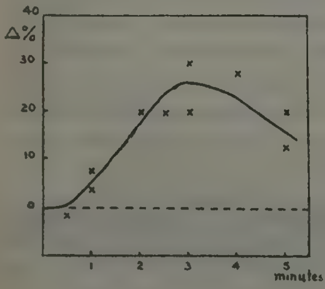

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Figures 2—8

The stimulation of germination by various periods in various thermostats: (2 at 30°, 3 at 37°, 4 at 45°, 5 at 50°, 6 at 60°, 7 at 80°, 8 at 90°) after 12 hours imbibition at 22° and further germination at 22° for 36 hours. The percentages of germination above controls are given.

The seeds began to respond to the temperature change after 4 hours of imbibition and had reached a level maximum of sensitivity at the 12th hour. The germination percentage for each treatment was determined. The difference between this and the control dishes, continuously kept at 22°C, was obtained. The stimulation obtained for each alternate temperature was plotted against the time at the alternate

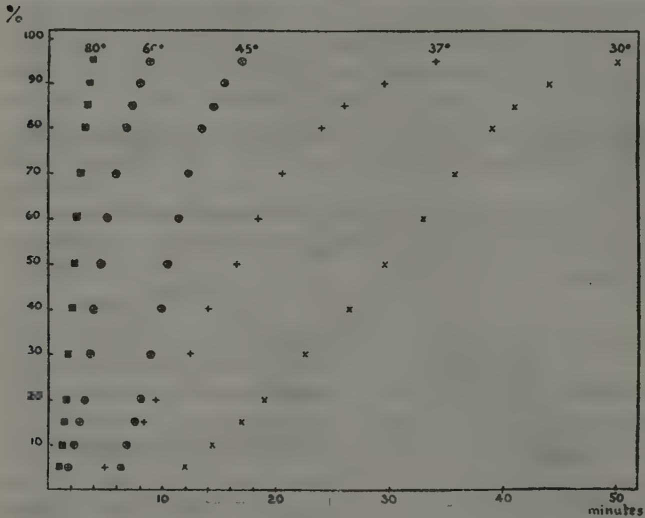

Figure 9
The stimulation of germination expressed as percentages of the maximal stimulation obtained in each thermostat, plotted against time in the thermostat. The temperatures are indicated in the curves.

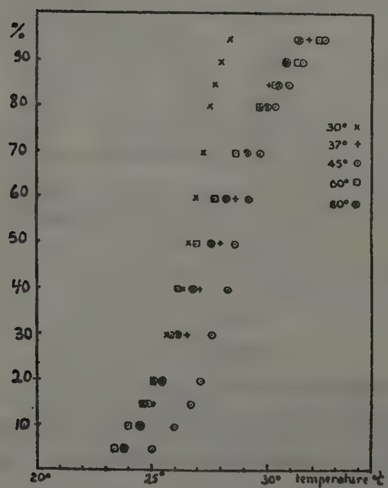

Figure 10
The same stimulation percentages as in Figure 9 plotted here against the maximal temperature in the dishes (see Figure 1).

temperatures. Best fitting curves were drawn by hand (Figures 2—8). The maximal stimulation obtained was that determined from these curves and are given in Table I. Maximal stimulation of germination was about the same in the different thermostats.

TABLE I

The relationship between maximal stimulation, above control, obtained and the alternate temperature used to obtain it. The values were obtained from Figure 2—8

Temp. of thermostat	*Maximal Δ% stimulation*
30°	32
37°	30
45°	24.5
50°	23.5
60°	20
80°	26.0
90°	26.5

The % germination stimulation at various times in the incubator (Figures 2—8) were then expressed as a percentage of the maximal stimulation in the same incubator, the latter being taken as 100%. These were plotted against the time in the incubator. (Figure 9). The maximum temperature obtained for each point in Figure 9 was deduced from Figure 1. The percentage stimulation was then plotted against this temperature. (Figure 10). It will be seen that all the curves are essentially the same. Stimulation correlates markedly with the maximal temperature in the dishes.

DISCUSSION

It will be seen that there is no correlation at all between stimulation and the rate or duration of the temperature change (Figure 1 and 9). Stimulation does show good correlation with the maximal temperature change (Figure 10). In all the thermostats tested, 50% stimulation was attained at about 27.5°C.

It appears therefore that dormancy breaking cannot be ascribed to a momentary change in the concentration of some metabolite, as suggested by Toole et al. (1955). The following interpretation is suggested : There are critical temperatures for some seeds, which, when exceeded cause them to germinate. The temperature dependence of the stimulation is the result of the distribution of these critical values in the seed-population. From Figure 10 it will be seen that here the critical temperatures are between 23°C to 32°C with 27.5°C as the median value. A critical dependence on temperature alone indicated a physio-chemical change of state, which depends only and sharply on temperature. Such a change must have a very high positive entropy, which means that it involves a highly organized structure, as for example solid or gel states.

If the responding seeds are considered as a uniform population then the fraction that germinates after a certain treatment is the fraction p of the whole change caused by the temperature treatment. From the dependence of p on temperature we can calculate the important thermodynamic values of the reaction.

Assuming $p/(1-p) = K$, where K is the "equilibrium" constant, then $RT \ln K = -\Delta F = \Delta H - T\Delta S$, where ΔF is the free energy change, ΔS is the entropy change, ΔH is the enthalpy or heat change, T is the absolute temperature, and R is the gas constant.

To obtain the ΔS and Δ H values, we used the van't Hoff equation $d\ln K/d(1/t) = \Delta H/R$.

The expression log $p/(1-p)$ which is equal to log $\Delta\%/(\Delta\%\ \text{max.} - \Delta\%)$ was calculated and plotted against $1/T$ (Figure 11). From the slope a ΔH value of + 70,000 cal/mole and a ΔS value of + 235 cal/deg. mole were calculated. These values fall nito the range of those calculated from protein denaturation data and thermal killing of various living cells (Johnson et al. 1954).

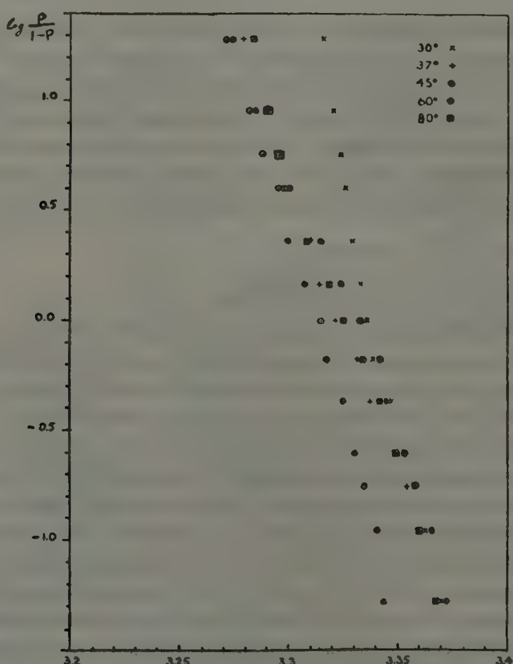

Figure 11

The logarithms of the stimulation percentages plotted against the reciprocal of the absolute temperature in the dishes.

This suggests the following interpretation of the results:

In lettuce seeds there is an organized structure or a complex macromolecular arrangement that prevents germination in a fraction of the seeds. This structure is destroyed rapidly by elevating the temperature. The destruction is an irreversible one, or allows an irreversible process to take over and initiate the germination.

The nature of this structure is unknown. It may be an inactive precursor of an enzyme that liberates the enzyme following the temperature rise. Similar cases have been reported (Johnson et al. 1954). Alternatively, the structure may be a membrane separating certain reactants within the seed. Destruction of the membrane or a change in its permeability following the temperature change allows the reactants to mix, and germination can be initiated.

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REFERENCES

1. Crocker, W. and Barton, L. V., 1953, *The Physiology of Seeds,* Chronica Botanica, Waltham, Mass.
2. Johnson, F. H., Eyring, H. and Polissar, M. J., 1954, *The Kinetic Basis of Molecular Biology,* J. Wiley and Sons, New York.
3. Morinaga, T., 1926, The effect of alternating temperatures on germination, *Am. J. Bot., 13,* 141—58.
4. Samish, R. M., 1954, Dormancy in woody plants, *Ann. Rev. Pl. Phys., 5,* 183.
5. Sussman, A. S., 1956, Metabolic aspects of Neurospora activation and germination, *Pl. Phys., 31,* 126.
6. Toole, E. H., Toole, V. K., Borthwick, H. A. and Hendricks, S. D., 1955, The interaction of temperature and light in germination, *Pl. Phys., 30,* 473.

THE INTERACTION OF THIOUREA AND COUMARIN IN GERMINATION AND GROWTH OF LETTUCE

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ABSTRACT

The joint action of thiourea and coumarin on germination and growth of lettuce is described. The two compounds show interaction in their effect on germination. This is marked by a change in sensitivity of the seeds to thiourea in the presence of coumarin. Thiourea and coumarin also show interaction in their effect on growth of roots and hypocotyls of lettuce seedlings. These interactions are discussed in the light of previous findings on the action and metabolism of thiourea and coumarin. It is suggested that the interaction in germination is related in some way to the oxidative destruction of coumarin.

Coumarin and thiourea are known to effect the germination of lettuce seeds. Thiourea abolishes the light dormancy of light sensitive varietes while coumarin induces light dormancy in light indifferent seeds, (Thompson and Kosar 1939, Nutile 1945, for other references see Evenari 1956).

The effect of thiourea on germination and growth of lettuce has been previously described (Poljakoff-Mayber, Mayer and Zacks 1958). Coumarin is known to affect the growth of plants (Goodwin and Taves 1950, Thiman and Bonner 1944, Tarragan 1953, Audus and Quastel 1947, Avers and Goodwin 1956). Coumarin and thiourea have opposite effects on germination. It was therefore of interest to study the effect of coumarin on growth of lettuce seedlings and also to determine whether there was some interaction if the two substances are applied together to lettuce seed. Some indication of such an interaction has been previously found (Mayer and Evenari 1952). The presence or absence of such interaction in this case is of special interest, as it may indicate to what extent different metabolic processes are involved in the action of the two compounds (Evenari 1957).

METHODS

Lettuce seeds variety Grand Rapids were used throughout the experiments. They were obtained from the Ferry Morse seed growers, California. Germination and growth were determined as previously described. (Poljakoff-Mayber, Mayer and Zacks 1958). Germination was carried out at 26°C in the dark.

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The statistical treatment of the results was designed to separate the treatment means into homogenous groups in order to test main effects of coumarin and thiourea and their interaction. The methods of separation were according to Tukey 1949 and Duncan 1955. An analysis of variance according to a two-way classification has not been used because of a lack of orthogonality between the various parts of the total "sum of squares" of deviations. This was due to the unequal number of replications in each treatment and unequal number of seeds in the replications (Kendall 1952). The variates subject to this analysis were $y = 2 \text{ arc sin } \sqrt{p}$ for the percentage germination p. As regards hypocotyl and root length the mean values of the replications in each treatment were used.

RESULTS

The effect of various concentrations of thiourea in the presence of coumarin at three different levels, 1, 2 and 5 mg% (i. e. 0.68, 1.36 and 3.4×10^{-4} M) are given in Tables I, II and III. These tables show the results for germination root length

TABLE I

The effect of thiourea and coumarin on the mean germination percentage of lettuce seed germinated for 72 hours at 26°C in the dark

Thiourea conc. M \ Coumarin conc.	0	1mg%	2mg%	5mg%
0	33.3	2.7	0.8	0.1
1×10^{-2}	57.8	15.6	8.5	0.05
2.5×10^{-2}	59.1	34.3	13.8	2.7
5×10^{-2}	63.4	14.4	3.6	0

TABLE II

The effect of thiourea and coumarin on the mean root length (in mm) of lettuce seedlings at 26°C in the dark 72 hours after germination

Thiourea conc. M \ Coumarin conc.	0	1mg%	2mg%	5mg%
0	12.6	3.7	0.9	0.3
1×10^{-2}	9.3	1.9	1.2	0.4
2.5×10^{-2}	4.2	1.7	1.2	0.9
5×10^{-2}	2.1	1.4	0.7	0

TABLE III

The effect of thiourea and coumarin on the mean hypocotyl length (in mm) of lettuce seedlings at 26°C in the dark after 72 hours of germination

Thiourea conc. M \ Coumarin conc.	0	1mg%	2mg%	5mg%
0	7.5	2.6	0.8	0.3
1×10^{-2}	4.1	1.9	1.4	0.1
2.5×10^{-2}	4.7	1.4	1.8	0.8
5×10^{-2}	2.6	1.1	0.7	0

TABLE IV

Homogenous groups of treatment means for germination for each concentration of thiourea with all coumarin concentrations (IV a) and vice versa (IV b)

TABLE IV a

Thiourea conc. $\times 10^{-2}$ M	Conc. of coumarin mg% in a homogenous group of means	95% confidence limits of means of the homogenous groups: upper	lower
0	0	38.5	28.1
	1	3.8	1.5
	2	1.3	2.0
	5	0.2	0
1.0	0	61.4	54.1
	1	19.05	12.1
	2	13.7	3.2
	5	0.1	0
2.5	0	70.4	47.7
	1	38.8	30.1
	2	18.9	8.3
	5	4.9	0.5
5.0	0	71.2	55.5
	1	17.8	10.9
	2	6.5	0.6
	5	0	0

TABLE IV b

Coumarin conc. in mg%	Thiourea conc. $\times 10^{-2}$ M in a homogenous groups of means			95% confidence limits of the mean groups: upper	lower
0	0	2.5	5.0	65.4	55.4
		0		33.9	27.7
1		2.5		38.9	29.5
	1.0		5.0	17.8	12.0
		0		3.8	1.2
2	1.0		2.5	13.9	5.5
	0		5.0	1.8	0.2
5		2.5		4.6	0.3
	0	1.0	5.0	0.2	0

and hypocotyl length respectively. Table IV shows the results giving the 95% confidence limits and the homogeneous groups of treatments for germination. Tables V and VI give the corresponding results for root length and hypocotyl length. The mean values of the results, if not significantly different for any two concentrations, are grouped together as for example 1 and 2 mg% coumarin with 2.5×10^{-2} M thiourea for hypocotyl length. (Table VI).

TABLE V

The homogenous groups and treatment means for root length for each concentration of thiourea with all coumarin concentrations (V a) and vice versa (V b)

TABLE V a

Thiourea conc. × 10^{-2} M	Conc. of coumarin mg% in a homogenous group of means	95% confidence limits of means of the homogenous groups upper	lower
0	0	13.34	11.86
	1	4.56	2.79
	2	1.39	0.474
	5	0.474	0.04
1.0	0	9.74	8.75
	1	2.61	1.29
	2	1.54	0.89
	5	1.06	0
2.5	0	4.45	3.85
	1	2.03	1.34
	2 5	1.45	0.54
5.0	0	2.45	1.65
	1	1.67	1.19
	2	1.04	0.29
	5	0	0

TABLE V b

Coumarin conc. in mg%	Thiourea conc. × 10^{-2} M in a homogenous groups of means	95% confidence limits of mean of the homogenous group upper	lower
0	0	12.84	12.36
	1.0	9.75	8.75
	2.5	4.45	2.85
	5	2.45	1.65
1	0	4.57	2.77
	1.0 2.5 5.0	1.99	1.39
2	0 1.0 2.5 5.0	1.23	0.71
5	2.5	1.27	0.47
	0 1.0 5.0	0.43	0.03

TABLE VI

Homogenous group of treatment means for hypocotyl length for each concentration of thiourea with all coumarin concentrations (VI a) and vice versa (VI b)

TABLE VI a

Thiourea conc. × 10^{-2} M	Conc. of coumarin mg% in a homogenous group of means	95% confidence limits of means of the homogenous groups upper	lower
0	0	8.54	6.36
	1	3.28	1.88
	2	1.16	0.43
	5	0.56	0.04
1.0	0	4.49	3.62
	1	2.93	1.76
	2	1.70	1.04
	5	0.45	0
2.5	0	5.12	4.26
	1 2	1.43	1.167
	5	1.163	0.46
5.0	0	3.16	2.11
	1	1.33	0.92
	2	0.99	0.31
	5	0	0

TABLE VI b

Coumarin conc. in mg%	Thiourea conc. $\times 10^{-2}$ M in a homogenous groups of means	95% confidence limits of mean of the mogenous group upper	lower
0	0	8.57	6.33
	1.0	4.73	4.65
	2.5	4.04	4.01
	5.0	3.17	2.09
1	0 1.0	2.92	1.88
	2.5 5.0	1.40	1.12
2	1.0 2.5	1.54	1.10
	0 5.0	1.04	0.48
5	2.5	1.17	0.45
	0 1.0 5.0	0.36	0.04

The results for the effect of thiourea alone will not be analysed here as they have been described in detail previously (Poljakoff-Mayber, Mayer and Zacks 1958).

Tables I and IV show that germination percentage decreases steeply with increasing coumarin concentration. Increasing thiourea concentrations increase the germination percentage as already reported for this lot of seeds (Poljakoff-Mayber, Mayer and Zacks 1958). When thiourea and coumarin are applied jointly an interaction is evident. The percentage germination is higher than in coumarin but lower than in thiourea. The optimal thiourea concentration for reversing the inhibition of coumarin is 2.5×10^{-2} M. The percentage germination at thiourea concentration of 1 and 5×10^{-2} M differ significantly only at 2mg% coumarin.

Table II and V show that there is no clear interaction of the two substances in their effect on root length at most concentrations. Both increasing thiourea and coumarin concentrations result in inhibition of root growth. If applied jointly, however, at 5mg% coumarin and 2.5×10^{-2} M thiourea, thiourea to some extent reverses the inhibition of coumarin, showing interaction to occur.

Tables III and VI show that in their effect on hypocotyl length the two substances again show interaction. The results resemble those obtained for root length. In the latter, however, interaction was apparent only at 5mg% coumarin. Hypocotyl length shows interaction at both 5 and 2mg% coumarin with 2.5×10^{-2} M thiourea.

DISCUSSION

As can be seen from the results, thiourea and coumarin have opposite effects on germination when applied separately. When applied jointly, this is still apparent. However, a shift towards lower concentrations of thiourea causing greatest stimulation appears. This shift provides evidence for interaction between two compounds. Previously it has been shown that lettuce seeds variety Grand Rapids of a different source have different sensitivity to thiourea. Here coumarin apparently can change the sensitivity of seeds to thiourea, in addition to inhibiting their germination.

The previously reported difference in sensitivity to thiourea was correlated with

the dormancy of the seeds, more dormant ones being more sensitive and showing an optimum concentration in the thiourea response curve. Coumarin induces a form of dormancy in lettuce seeds which can be broken by light. This is very suggestive of a similar mechanism causing a change in sensitivity to thiourea. A change in sensitivity to thiourea, in thiourea, in the seeds with an optimal concentration for stimulation has several implications. One of these is that certain processes which were previously insensitive to thiourea are being inhibited by it in the presence of coumarin, when a sufficiently high internal thiourea concentration is reached. This must be considered in the light of our previous findings. The internal thiourea concentration increases both with time and with external thiourea concentration (Mayer 1956). This increase is particularly marked with time, the internal concentration increasing rapidly after 48 hours of germination. Coumarin is known to be destroyed by the seeds; this destruction increases with time of germination (Mayer 1953). It seems quite possible that the interaction is between the coumarin destroying system and thiourea. Thiourea is known to stimulate certain oxidative sytsems in vivo (Poljakoff-Mayber 1953, Mayer 1954). If coumarin is destroyed by an oxidative process, then thiourea might well affect germination in the presence of coumarin by affecting the destruction of the latter.

The optimal stimulation by thiourea in joint action could be accounted for as follows: Thiourea probably affects more than one process in the germination of seeds. Normally, the balance of these processes is stimulation, some being inhibited, others stimulated. The balance of stimulation to inhibition changes with the thiourea concentration, favouring inhibition, as the concentration increases. This is indicated by the behaviour of different seeds towards thiourea (Poljakoff-Mayber, Mayer and Zacks 1957). If coumarin antagonises the stimulatory process it would cause a shift in the balance from stimulation to inhibition. A balance favourable to inhibition would then be expected to occur at a lower thiourea concentration. Hence, a response curve with an optimum thiourea concentration appears.

Germination precedes growth, and therefore thiourea stimulation of germination precedes its inhibition of growth. This seems clearly correlated with the increasing internal thiourea concentration. Germination stimulation occurs while the internal thiourea concentration is still low, growth inhibition occurs when it rises considerably. It is not clear whether thiourea affects germination and growth through the same process, but at different concentrations, or through entirely different processes.

Coumarin inhibits both germination and growth, but here again it is not clear whether the same or different processes are involved.

In some cases the inhibition of growth by the two substances is additive. At one thiourea concentration (2.5×10^{-2} M) however, there is interaction between thiourea and certain coumarin concentrations. It is of interest that this is the same thiourea concentration which also shows particularly marked interaction with coumarin in

their effect on germination. The significance of this interaction at a specific thiourea concentration is quite unclear at present.

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REFERENCES

1. Audus L. J. Quastel, J. 1947, Coumarin as a selective phytocidal agent, *Nature, 159,* 320—24.
2. Avers, Ch. J., and Goodwin, R. H., 1956, Studies on roots. IV. Effect of coumarin and scopoletin on the standard root growth pattern of Phleum pratense, *Am. J. Bot., 43,* 612.
3. Duncan, D. B., 1955, A multiple Range and multiple F-test, *Biometrics, 11,* 1.
4. Evenari, M., 1956, Seed germination, in *Radiation Biology, 3,* p. 519, McGraw Hill, New York.
5. Evenari, M., 1957, The physiological action and biological importance of germination inhibition. SEB Symposium XI. The biological action of growth substances, Cambridge Univ. Press.
6. Goodwin, R. H. and Taves, C., 1950, The effect of coumarin derivatives on the growth of Avena roots, *Am. J. Bot., 37,* 224—31.
7. Kendall, M. G., 1952, The advanced theory of statistics, Charles Griffin and Co. Ltd., London.
8. Mayer, A. M., 1953, Polyphenol oxidases of lettuce seed and their inhibition, *Enzymologia, 17,* 277.
9. Mayer, 1953, Quantitative aspects of the behaviour of coumarin as a germination inhibitor, *Physiol. Plant., 6,* 413—424.
10. Mayer, A. M., 1956, The action of thiourea as a germination stimulator, *J. Exp. Bot., 7,* 93.
11. Mayer, A. M. and Evenari, M., 1952, The influence of two germination inhibitors (coumarin and 2,4D) on growth in conjunction with thiourea and cysteine, *Bull. Res. Counc. of Israel, 1,* 125—29.
12. Nutile, G. E., 1945, Inducing dormancy in lettuce seeds with coumarin, *Plant Phys., 20,* 433.
13. Poljakoff-Mayber, A., 1953, Peroxidase activity in germinating lettuce seeds, *Enzymologia, 16,* 122.
14. Poljakoff-Mayber, A., Mayer, A. M. and Zacks, S., 1958, Interaction of indolyl acetic acid and thiourea in germination and growth of lettuce, *An. Bot.* (in press).
15. Tarragan, M., 1953, The effect of coumarin on the growth of tomato roots in cultures, *Bull. Res. Counc. of Israel, 3,* 254—5.
16. Thiman, K. V. and Bonner, W. D., 1944, Inhibition of plant growth by proto anemonin and coumarin and its prevention by B. A. L., *Proc. Nat. Acad. Sc., 35,* 272—276.
17. Thompson, R. C. and Kosar, W. F., 1939, Stimulation of germination of dormant lettuce seeds by sulphur compounds, *Plant. Phys., 14,* 567.
18. Tukey, J. W., 1949, Comparing individual Means, *Biometrics, 5,* 99.

RESPIRATION STUDIES WITH LETTUCE SEEDS IMBIBED FOR VERY LONG PERIODS

In a previous paper[1] it was reported that the respiration rate of lettuce seeds declined after 24 hours in 26°C, if imbibed under conditions unfavourable for germination, i. e. darkness of far red irradiation. These fully imbibed seeds, which did not germinate during the above mentioned period, lose their photoblastism as the imbibition time is prolonged.. After twenty four days, they no longer react to red light. They can be brought to full germination, however, if given a cold temperature treatment of two days in the refrigerator. This shows that although their viability is not impaired, long imbibition under conditions unfavourable for germination changes their physiological behaviour as shown by their reaction to some external factors. Some authors regard this phenomenon to be a sort of "secondary" or "induced dormancy". In the light of this change in the physiological behaviour of the seeds it was of interest to investigate the changes, in the rate of their respiration and also the change in concentration of some metabolites, during such long imbibition periods.

Lettuce seeds, variety Grand Rapids, were used in these experiments. They were germinated in Petri dishes, on filter paper in the dark at 26°C. After 24 and 48 hours, all the germinated seeds were taken out in blue light. The non-germinated seeds were periodically transferred to new dishes in the dark. This minimised fungal infection.

Respiration measurements were conducted according to the conventional Warburg technique, using the direct method[2]. The sugar and lipid content were measured as previously described[3].

The results of the respiration measurements, with seeds imbibed for various lengths of time are summarized in Figure 1. These results are expressed as μl gas, exchanged per hour by a number of seeds equivalent to 100 mg of initial dry weight. As it is seen from Figure 1, the respiration rate continues to decline until, after 15

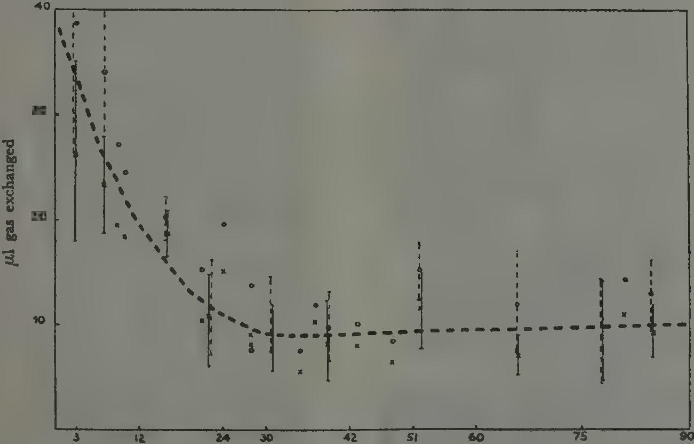

Figure 1

Respiration rate of lettuce seeds after various periods of imbibition. The imbibition was carried out under conditions unfavourable for germination.

Some characteristic standard deviations are shown in the curve. In view of the obvious overlap of the standard deviations for O_2 and CO_2 measurements, a free hand curve was drawn between all the points.

o — oxygen uptake   x — CO_2 evolution.

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days of imbibition, the respiration rate is very near that of dry seeds.

No measurable changes in lipid content were detected. There was no increase in glucose content, although normally such an increase accompanies germination [3]. A measurable decrease in sucrose content was evident only during the first three days of imbibition. After this period there was a tendency for the sucrose content to decrease slightly, but this could not be firmly established. These facts would be in agreement with the view that sucrose is the main substrate oxidized during the imbibition period.

It seems as though during the long imbibition, under conditions unfavourable for germination, the seeds do not become fully active and return to some form of dormancy. The metabolic processes awakened by the hydration of the tissues, return to their latent rate, although no apparent dehydration takes place. The mechanism causing this "metabolic reversal" is as yet unclear.

Results of similar respiratory behaviour, were reported by other authors for various varieties of lettuce seeds, Hansen [4], Big Boston [5] and Grand Rapids[6]. For Xanthium[7], Amaranthus [8] and Impatiens [8], similar behaviour has been reported. In all these cases, if germination does not occur, there is a break in the respiration curve, at which a steady drop begins, leading to very low rates of respiration. The time of this break coincides more or less, with the end of the germination rush observable under conditions permitting germination to occur. In the period up to the turning point, the respiration curves are identical for seeds that germinate and for those that do not germinate. Then the seeds that do germinate, show a steep rise in their germination rate 1, 4, 5, while in those that fail to germinate, due to unfavourable external conditions, the respiration rate declines (ref. 1, 6 and the present results).

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REFERENCES

1. Evenari, M., Neumann, G. and Klein, S., 1955, The influence of red and infra-red light on the respiration of photoblastic seeds, *Phys. Plant., 8,* 33—47.
2. Umbreit, W. W., Burris, R. H. and Stauffer, J. F., 1951, *Manometric Technique and Tissue Metabolism,* Burgess Publishing Co., Minneapolis.
3. Poljakoff-Mayber, A., 1952, Changes in metabolism of lettuce seeds during germination and its inhibition, *Pal. J. Bot. Jer. Ser., 5,* 180.
4. Leggatt, C. W., 1948, A contribution to the study of dormancy in seeds, *Canad. J. Res., C. 26,* 194—217.
5. Hagen, C. E., Borthwick, H. A. and Hendricks, S. B., 1954, Oxygen consumption of lettuce seed in relation to photocontrol of germination, *Bot. Gaz., 115,* 360—364.
6. Evenari, M., Neumann, G. and Poljakoff-Mayber, A., 1958, The respiration of lettuce seeds germinated at a high temperature, *Bull. Res. Counc. of Israel, 6D,* 106.
7. Ota, J., 1925, Continuous respiration studies of dormant seeds of *Xanthium, Bot. Gaz., 80,* 288—299.
8. Barton, L. V., 1945, Respiration and germination studies of seeds in moist storage, *Ann. N. W. Acad. Sci., 56,* 185—208.

CHANGES IN THE ASCORBIC ACID CONTENT OF GERMINATING LETTUCE SEEDS

It has been previously shown in this laboratory that in lettuce seed various oxidative pathways operate during germination, depending upon the external conditions [1]. The existence of the cytochrome oxidase system was also shown [1].

In view of these findings, the possible existence of other terminal oxidases was investigated. Polyphenol oxidase has been shown to operate [2]. In pea tissue the ascorbic acid — ascorbic acid oxidase system has been demonstrated by Mapson and Moustafa [3]. It was of interest, therefore, to see whether such a system could operate in lettuce seeds. As a first step, the occurrence of ascorbic acid in the germinating seed was investigated.

Lettuce seeds variety Grand Rapids were used throughout and germinated in Petri dishes on filter paper. Ascorbic acid was determined by the colorimetric dichlorophenol indophenol method [4]. The ascorbic acid content of seeds germinated at 20°C and 26°C is given in Table I.

Both at 20°C and 26°C the ascorbic acid content rises most rapidly between 48 and 72 hours, when germination is actually already completed. The effect of the germination inhibitor coumarin, on the ascorbic acid content is shown in Table II.

TABLE I

Changes in ascorbic acid content of lettuce seeds during germination
The light treatment was 500 f. c. white light for 1 minute given after 2 hours of germination

Germination conditions	*Age in hrs.*	20°C *mg ascorbic acid/100 g seeds* *Mean*	*S. D.*	*Germ. %*	26°C *mg ascorbic acid/100 g seeds* *Mean*	*S. D.*	*Germ. %*
Dark	0	2.94	0.15				
	24	0.64	0.23	40	1.0	0.20	40
	48	0.90	0.17		1.04	0.36	
	72	2.06	0.28		1.6	0.23	
Light	24	0.96	0.17		1.16	0.16	
	48	1.04	0.14	60	1.8	0.14	100
	72	2.40	0.33		2.7	0.60	

TABLE II

Ascorbic acid content of seeds germinated in 1 mg % coumarin at 26°C

Germination conditions	*Age in hrs.*	*Ascorbic acid content mg ascorbic acid/100 g seeds* *Mean*	*S. D.*	*Germ. %*
Dark	0	1.7	0.16	
	24	0.86	0.16	
	48	0.94	0.15	4—8
	72	1.0	0.13	
Light	24	1.0	0.28	
(as in Table I)	48	1.0	0.24	60—70
	72	1.2	0.23	

Coumarin clearly depresses ascorbic acid formation in the light. In the dark, its effect is more complicated, causing an initial rise at 48 hours, followed by a decrease after 72 hours, despite the lack of germination and growth. In seeds which germinated normally the ascorbic acid content rises as growth proceeds. In the light, coumarin markedly depresses ascorbic acid formation and growth, although the germination percentage is high.

No conclusion can be drawn about the functioning of the ascorbic acid — ascorbic acid oxidase system. However, the small amount of ascorbic acid present may be enough to permit the functioning of this electron transport system.

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REFERENCES

1. MAYER, A. M., POLJAKOFF-MAYBER, A. and APPLEMAN, W., 1957, *Physiol. Plant., 10,* 1.
2. MAYER, A. M., 1954, *Enzymologia, 16,* 227.
3. MAPSON, L. M. and MOUSTAFA, E. M., 1956, *Biochem. J., 62,* 248.
4. *Methods of vitamin assay,* 2nd ed. Association of Vitamin Chemists Inc.

VERNALIZATION EFFECTS IN MANGOLD AND SUGAR-BEET UNDER THERMAL CONDITIONS UNFAVOURABLE TO FLOWERING

Vernalization of beet seeds with a view to inducing flower-head formation was attempted by a number of workers with a varying measure of success. A comprehensive review of literature pertaining to beet vernalization is included in a recent monograph on sugar-beet [1]. It would appear that temperature constitutes a decisive factor in the 'photothermal induction' of flowering 2, 3, and that low temperature treatment, while capable of accelerating flowering when applied at the seeding stage [4], becomes increasingly effective as the cold treatment

is delayed to more advanced stages of development [5]. It is maintained that bolting is promoted by vernalization, provided that the vernalized seeds are planted in an environment favourable enough for induction of flowering to continue [3]. Devernalization effects due to high temperature after planting have been described by several authors.

Poor yields of beet seed are usually obtained by Israeli seed producers in seasons following a mild winter, even in the cooler hilly regions. Experiments of a preliminary nature were therefore carried out in order to ascertain whether bolting can be promoted by seed vernalization, when subsequent development takes place under conditions of excessively high temperature combined with a favourable photoperiodic regime, such as would be brought about by very late planting. Duplicate samples containing several hundred seeds each of two mangold and two sugar-beet varieties were sterilized with a 10 per cent solution of commercial formalin and soaked overnight in water. The seeds were then placed in moist canvas wrappings in closed plastic containers and allowed to germinate at a constant temperature of 22°C for periods of five and seven days respectively, before being introduced into a refrigerator main-low temperature treatment, the seeds were planted out in field plots at Ein Karem near Jerusalem on March 26th, 1954. Bolting records as presented in table I were taken on June 2nd, and identical results were recor-tained at 2.5 ± 0.5°C. After $4\frac{1}{2}$ months of ded three weeks later. The extreme minimum air temperatures at Ein Karem for the months of March, April and May were 3.5°, 4.0°, 7.5°C, and the respective maxima were 32.0°, 30.4°, 36.5°C. The length of day increased during the recorded growth period from $12\frac{1}{2}$ hours to the longest photoperiod at the latitude of Jerusalem, i. e. $14\frac{1}{2}$ hours.

TABLE I

Effect of seed vernalization on the percentage of bolters

Variety	*Imbibition period prior to vernalization* 6 days	8 days	*Non-vernalized controls*
Lord Warden	0.42	0.49	0.00
Danish Sludstrop	0.00	0.00	0.00
Coons	0.00	0.58	0.00
Pedigree	5.55	17.95	0.00

In the case of Pedigree sugar-beet, the effect of vernalization proved highly significant (at 1% level), while the difference due to duration of imbibition fell in this variety just short of significance at the 5% level. It appears that, at least in some varieties, bolting can be secured by prolonged low-temperature treatment of the germinated seed, even when thermal conditions during the growing period are such as would normally preclude flower induction. It seems likely that an adequately replicated experiment would also confirm the significance of the differences brought about by the extent of imbibition.

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REFERENCES

1. Margara, J., 1954, Problèmes que pose l'amélioration de la betterave sucrière en France. *Ann. Amélior. Plantes*, *4*, 147.
2. Chroboczek, E., 1934, A study of some ecological factors influencing seed-stalk development in beets (*Beta vulgaris* L.). *Mem. N. Y. (Cornell) agric. Exp. Sta.*, *154*.
3. Owen, F. V., Carsner, E. and Stout, M., 1940, Phototermal induction of flowering in sugar beets. *J. agric. Res.*, *61*, 101.
4. Beljdenkowa, A. F., Koriakina, V. F. and Smetannikowa, A. I., 1945, How seed from some biennial vegetable crops can be produced in one year (Russian). *Sovetsk. Bot.*, *13* (5), 29.
5. Erdmann, K., 1951, Verfahren zur Erzielung von vollvertigen Samenträgern der *Beta*-Rübe im ersten Vegetationsjahr mit Hilfe von Kälte-Behandlung. 2. Mitteilung. *Züchter*, *21*, 110.

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